Ontogenetic development underlies population response to mortality [post-print]

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35   **Keywords:** Biomass overcompensation, *Daphnia pulex*, development, harvesting, ontogenetic
36   asymmetry, stage-structure.

37   ABSTRACT

38   Understanding demographic responses to mortality is crucial to predictive ecology. While

39   classic ecological theory posits reductions in population biomass in response to extrinsic

40   mortality, models containing realistic developmental change predict the potential for

41   counterintuitive increase in stage-specific biomass, i.e., biomass overcompensation. Patterns of

42   biomass overcompensation should be predictable based on differences in the relative energetic

43   efficiencies of juvenile maturation and adult reproduction. Specifically, in populations where

44   reproduction is the limiting process, adult-specific mortality should enhance total reproduction

45   and thus juvenile biomass. We tested this prediction by inducing an array of stage-specific

46   harvesting treatments across replicate populations of *Daphnia pulex*. In accordance with

47   reproductive regulation, the greatest biomass response occurred in the juvenile *Daphnia* stage and

48   this response occurred most strongly in response to adult mortality. Nevertheless, we failed to

49   detect significant biomass overcompensation and instead report largely compensatory effects. In

50   total, our work demonstrates that knowledge of population structure is necessary to accurately

51   predict population dynamics, but cautions that further research is needed to illuminate the factors

52   generating over-compensatory versus compensatory responses across natural populations.

INTRODUCTION

54   Understanding how populations respond to perturbations, such as changes in mortality or

55 resource productivity, is central to predicting ecological dynamics (Nicholson 1957, Turchin

56 2003, Yang et al. 2008). Our understanding of such responses traditionally derives from

57 unstructured models which assume reproduction and mortality are the only processes underlying

58 population change. Yet, ontogenetic development is ubiquitous within natural populations

59 (Werner and Gilliam 1984, Rudolf and Lafferty 2011, Miller and Rudolf 2011), and both

60 reproductive output and mortality risk change as individuals grow (i.e., size- or stage-dependent

61 life history traits; de Roos and Persson 2013). Accordingly, theory incorporating size or stage-

62 dependence produces an array of population dynamic patterns not possible in analogous

63 unstructured models, including biomass overcompensation, cohort cycles, emergent facilitation,

64 and alternative stable community states (de Roos et al. 2003, Miller and Rudolf 2011, de Roos

65 and Persson 2013, Huss et al. 2013, Schröder et al. 2014). While developmental variation has

66 been incorporated into increasingly complex community models (van de Wolfshaar et al. 2006,

67 Ohlberger et al. 2012, Leeuwen et al. 2014, Toscano et al. 2017), some of the most fundamental

68 and far-reaching predictions of this new theory, such as population responses to perturbation,

69 still require focused experimental testing. These tests are necessary to revise our understanding

70 of the basic processes driving population and community dynamics.

71   Perhaps the most important and far-reaching way in which unstructured and structured

72 model predictions diverge is regarding population responses to extrinsic mortality (de Roos et al.

73 2007), a widespread natural perturbation. Unstructured models generally predict reductions in

74 population density and biomass due to mortality, and this basic prediction is implicit in the vast

75 majority of classical ecological theory (but see (Abrams 2009, Schröder et al. 2014) for scenarios

76   in which unstructured models can produce positive responses to mortality, or "hydra effects"). In

77   contrast, structured models containing a realistic representation of the developmental process

78   predict, counterintuitively, that biomass can increase in response to mortality (de Roos and

79   Persson 2013, Schröder et al. 2014). The ultimate cause of such positive biomass responses is

80   ontogenetic asymmetry, or differences in the relative energetic efficiencies of developmental

81   stages (de Roos et al. 2007). For example, for fish populations, competition is generally more

82   intense within the reproductive adult stage versus within the juvenile stage (i.e., juveniles use

83   energy more efficiently), a scenario termed reproduction regulation (Persson and de Roos 2006,

84   Schröder et al. 2009). Here, biomass builds up within the adult stage due to a reproductive

85   bottleneck at normal background mortality levels, and increasing adult mortality is expected to

86   enhance juvenile fish biomass via increased reproduction. More generally, mortality acts to

87   release the energetically less efficient (i.e., regulatory) developmental stage from intra-stage

88   competition, thereby enhancing the biomass production of this stage. Thus, positive effects of

89   mortality on stage-specific biomass should be predictable based on the ontogenetic asymmetry of

90   a population (Persson and de Roos 2013). Empirical tests of such predictions can illuminate the

91   importance of developmental variation in governing natural population dynamics.

92   The magnitude of positive biomass responses to mortality within structured populations

93   should further depend on the stage-specificity of mortality. Using a general structured consumer-

94   resource model, de Roos et al. (2007) show that stage-specific biomass overcompensation is a

95   widespread response to mortality in populations characterized by ontogenetic asymmetry:

96   overcompensation occurs in response to both stage-independent and stage-specific mortality,

97   even when stage-specific mortality is induced within the non-regulatory stage (i.e., the stage

98   exhibiting overcompensation). Nevertheless, the magnitude of overcompensation varies across

99   these different types of mortality: overcompensation is greatest when mortality targets the

100 regulatory stage, intermediate when mortality is stage-independent, and least when mortality

101 targets the non-regulatory stage (de Roos et al. 2007). Though empirical studies testing for

102 biomass overcompensation are still rare (Schröder et al. 2014), only one study to our knowledge

103 has induced (stage-specific) mortality across different stages while monitoring biomass, rather

104 than density, responses (Schröder et al. 2009, 2014). Such studies are ultimately necessary to

105 pinpoint ontogenetic asymmetry in energetics as the property that allows for biomass

106 overcompensation (Schröder et al. 2014).

107   The goal of our study was to test for the occurrence of biomass overcompensation, a key

108 population dynamic phenomenon, as driven by developmental change. To accomplish this, we

109 harvested different developmental stages within lab populations of *Daphnia pulex* while

110 monitoring stage-specific and total population biomass and density responses over 7 *Daphnia*

111 generations. Evidence for ontogenetic asymmetry within *Daphnia* enables us to make clear

112 predictions regarding the effects of developmental variation on population dynamics. Intra-stage

113 competition appears stronger within adult versus juvenile *Daphnia* (i.e., reproductive regulation:

114 present study, Nilsson et al. 2010) and given this adult-dominated population structure, we

115 hypothesized that positive biomass responses to mortality would occur in the juvenile, rather

116 than the adult *Daphnia* stage. Furthermore, we predicted overcompensation would be strongest

117 in response to adult, rather than juvenile *Daphnia* mortality, because adult mortality most

118 directly reduces competition within this regulatory stage (de Roos et al. 2007). While our results

119 support the predicted stage-specificity of positive biomass responses, we detected largely

120 compensatory rather than overcompensatory biomass responses. Thus, our study demonstrates

121 the importance of ontogenetic development in population responses to perturbation but cautions

122 that more work is needed before biomass overcompensation is presumed a general phenomenon

123 in nature.

124 <center>METHODS</center>

125 *Study system*

126   We tested population responses to stage-specific mortality using replicate lab populations

127 of *Daphnia pulex. Daphnia* (Order: Cladocera) is a key herbivore and important prey for size-

128 selective predators within freshwater food webs (Brooks and Dodson 1965, Sarnelle 1993,

129 Boersma et al. 1996, Wagner et al. 2004). A number of studies have explored the role of

130 developmental processes in generating *Daphnia*'s well-known cyclic population dynamics (e.g.

131 McCauley et al. 1990b, 2008), establishing this genus as an important model regarding the

132 population dynamic consequences of ontogenetic stage structure.

133   We collected *Daphnia* from a small fishless pond in Huntsville, Texas and maintained

134 populations under high food conditions in the lab for several months prior to experiments.

135 Reproductively mature, female *Daphnia* produce clonal female offspring when environmental

136 conditions are favorable and ephippia (haploid eggs that are fertilized by males) when resources

137 are scarce. *Daphnia* size at reproductive maturity depends on food availability and temperature,

138 among other environmental variables (McCauley et al. 1990a, Gurney et al. 1990, Stibor 1992).

139 Within the present study (i.e., our lab environment), *Daphnia* reached reproductive maturity (i.e.,

140 began producing eggs) as small as 0.9 mm. Thus, we considered *Daphnia* below this size

141 threshold as juveniles and *Daphnia* above this size threshold as adults, though our findings do

142 not depend on the precise threshold value (Supplementary Fig. 1).

143 *Treatments*

<center>6</center>

144   *Daphnia* populations within our experimental setup are dominated by adult biomass in

145   the absence of extrinsic mortality (Fig. 2A, control treatment; see also Nilsson et al. 2010). For

146   reasons explained in the *Introduction*, mortality inflicted on adults (relative to mortality targeting

147   juveniles or stage-independent mortality) should produce the greatest positive biomass response

148   because it most directly reduces competition within the adult stage (de Roos et al. 2007).

149   Accordingly, our experimental design focused on adult harvesting.

150   We applied 4 treatments to replicate *Daphnia* populations (n = 4 replicates per

151   treatment): (1) a high level of adult-specific harvesting (instantaneous adult mortality rate: 0.07

152   per day); (2) a low level of adult-specific harvesting (instantaneous adult mortality rate: 0.04 per

153   day); (3) a high level of juvenile-specific harvesting (instantaneous juvenile mortality rate: 0.07

154   per day); and (4) a no-harvest control. Overcompensation occurs as a hump-shaped response

155   between biomass and mortality (de Roos et al. 2007, Nilsson et al. 2010), and thus we harvested

156   adult *Daphnia* at two different levels to increase our chances of capturing this hump-shaped

157   region. Furthermore, a previous study on *Daphnia* biomass overcompensation induced a single

158   high level of stage-independent mortality (instantaneous total mortality rate: 0.2 per day), which

159   the authors suggested could have overwhelmed any positive biomass response (Nilsson et al.

160   2010). Again, due to the hypothesized reproduction regulation of *Daphnia* populations, we

161   excluded a low-level juvenile-specific harvesting treatment from our design because we expected

162   juvenile mortality to induce the weakest biomass response.

163   *Experimental setup*

164   We ran the experiment in a semi-chemostat system. Our system used peristaltic pumps to

165   deliver algae continuously to microcosms housing replicate *Daphnia* populations. Each

166   cylindrical microcosm (3 L volume) featured four 63 μ nylon mesh-covered outflow holes at the

167   water surface to retain *Daphnia* but allow suspended particulate matter to pass through. This

168   flow-through setup creates semi-chemostat resource dynamics, an assumption of models in

169   which the conditions for biomass overcompensation have been explored (de Roos et al. 2008,

170   Nilsson et al. 2010, Huss and Nilsson 2011). Microcosms (n = 16) were arranged in 2 adjacent

171   spatial blocks and treatments were assigned randomly within each spatial block (2 replicates per

172   block). Microcosms within each spatial block shared the same peristaltic pump, algae stock tank

173   and drainage system.

174   We used a 3:1 mixture of natural and simulated pond water as the experimental medium

175   because our previous work has shown this mixture effective in maintaining *Daphnia* populations

176   over multiple generations. Natural pond water was collected monthly from a small pond in Sam

177   Houston National Forest, Texas. This pond water was then stored in a refrigerated room prior to

178   preparation for use in the experiment. Pond water preparation followed a 2-step purification

179   process including 10 μ vacuum filtration and 3 h autoclave sterilization. Artificial pond water

180   was created following the 'animal medium' recipe in Wyngaard and Chinnappa (1982). All

181   experiments were conducted in a temperature-controlled room (set at 22° C) under a 12:12 dark-

182   light cycle.

183   *Algae culture and delivery to microcosms*

184   The flagellated green alga *Chlamydomonas reinhardtii* was cultured in the lab and used

185   as the food resource for *Daphnia*. Algae (CC-1010 wild type mt+ [UTEX 90], *Chlamydomonas*

186   Resource Center) was grown in flasks containing TAP (Tris-Acetate-Phosphate) growth medium

187   under continuous light. Algae was harvested near peak density and centrifuged at 5000 rpm for 8

188   minutes. After centrifugation, TAP medium was discarded and replaced with simulated pond

189   water. We then measured algae cell density using a hemocytometer and added this concentrated

190   algae to stock tanks containing 16 L of the experimental medium. We adjusted the volume of

191   algae added to stock tanks to maintain a constant resource level (157,480 cells ml^{-1}) entering

192   microcosms throughout the experiment. Stock tanks containing magnetic stir bars were

193   continuously mixed over stir plates to keep algae suspended. Peristaltic pumps drew diluted

194   algae from stock tanks and delivered it (using 1.42 mm ID Tygon tubing) to experimental

195   microcosms (0.945 mL min^{-1} inflow rate, 0.019 h^{-1} dilution rate) housing *Daphnia* populations.

196   Algae stock tanks that fed microcosms were cleaned and replenished with fresh algae and

197   experimental medium every 48 h throughout the duration of the experiment.

198   *Experimental harvesting*

199   *Daphnia* were introduced to microcosms (50 individuals per microcosm) on 11

200   December 2017 and populations were allowed to increase and enter a regular pattern of cycling

201   before the start of harvesting treatments. *Daphnia* populations were harvested once per week

202   from 16 January 2018 until 27 March 2018 (11 total harvesting events). *Daphnia* generation time

203   within our semi-chemostat system is roughly 2 weeks and thus our experiment allowed for

204   approximately 7 *Daphnia* generations.

205   During each harvesting event we first stirred microcosm contents to homogenize

206   contents. Using a 50 mL Hensen Stempel zooplankton sampling pipette, we then removed either

207   50% of the microcosm volume (1500 mL, high level harvesting treatments) or 25% of the

208   microcosm volume (750 mL, low level harvesting treatment), depending on the assigned

209   treatment. For the control treatment we removed 750 mL of medium containing *Daphnia* and

210   followed the process for removing adults (see explanation below) but returned all contents to

211   control microcosms (i.e., a sham treatment).

212   To remove adult *Daphnia*, water from microcosms was filtered through 530 µ mesh that

213   retained adults but allowed juveniles to pass through. This mesh was then rinsed thoroughly to

214   capture adult *Daphnia*. The water containing any *Daphnia* that passed through the mesh was

215   returned to microcosms. To remove juveniles, water was first filtered through 530 µ mesh. This

216   mesh was rinsed to capture adult *Daphnia*, which were returned to microcosms. The water

217   containing juvenile *Daphnia* that passed through the 530 µ mesh was next filtered through 153 µ

218   mesh that retained juvenile *Daphnia* but allowed algae and resting eggs to pass through (also

219   returned to microcosms). This 153 µ mesh was then rinsed to capture juvenile *Daphnia.* All

220   *Daphnia* removed from microcosms were processed as detailed below (*Sampling*) to estimate the

221   amount and stage-specificity of *Daphnia* biomass removed by harvesting treatments.

222   *Sampling*

223   We sampled microcosms twice per week throughout the duration of the experiment.

224   Sampling events occurred on the first and third days after harvesting. For each sample,

225   microcosm contents were stirred, and 200 mL was removed using a 50 mL Hensen Stempel

226   zooplankton sampling pipette. We measured the total length of the first 30 *Daphnia* individuals

227   (or fewer if there were less than 30 individuals) within each 200 mL sample and counted eggs,

228   young in brood pouches, and resting eggs for reproductive females. Any additional *Daphnia* (>

229   30) within each 200 mL sample were counted. These samples were then returned to microcosms

230   (i.e., non-destructive sampling).

231   We applied length-weight regressions to derive *Daphnia* biomass densities. If the total

232   number of individuals per sample was less than 30, we applied a length-weight relationship

233   (Nilsson et al. 2010) to individual length measurements to estimate juvenile, adult and total

234   biomass per 200 mL sample. If the total number of *Daphnia* per sample was greater than 30

235 individuals, we used the size distribution from the 30 measured individuals to estimate the

236 biomass of unmeasured individuals and combined these biomasses to yield biomass estimates per

237 sample. To do this, we first calculated the proportion of individuals within 0.1 mm size classes

238 (ranging from 0.3-1.2 mm) out of the 30 measured individuals within each sample. We then

239 multiplied these proportions times the total number of unmeasured individuals, rounding down to

240 the nearest individual. Assuming that unmeasured individuals within each size class were of the

241 mean length (e.g., 0.45 mm in the 0.4-0.5 mm size class), we used these lengths to calculate the

242 biomass of unmeasured individuals. We applied this same basic scaling procedure to derive

243 estimates of stage-specific population densities per sample. Individuals ≥ 0.9 mm were

244 considered adults and individuals < 0.9 mm were considered juveniles in all calculations.

245 *Analysis*

246 Our experiment consisted of 11 weeks of treatments and 27 sampling events between 5

247 January 2018 and 13 April 2018. Two of the sampling events were conducted before treatments

248 began and four were conducted after treatments ceased. Analyses covered the 21 sampling events

249 during treatments and 2 sampling events after treatments had concluded to capture the effects of

250 the final harvesting event.

251 We tested stage-specific harvesting treatment effects on: (1) the amount of stage-specific

252 biomass removed from populations; (2) stage-specific and total population biomass and density;

253 and (3) reproductive responses. We examined the amount of stage-specific biomass removed

254 from populations to test the efficacy of harvesting treatments. We further tracked population

255 density responses, in addition to biomass responses, to provide deeper insight into population

256 responses to mortality (Schröder et al. 2014). Treatment effects on reproductive responses were

257 examined to explore potential mechanisms behind biomass and density responses to mortality.

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258   We calculated two reproductive response variables: average clutch size (a *per capita* measure)

259   and total reproductive output (a population-level measure). Average clutch size was calculated

260   by dividing the total number of eggs + offspring in brood pouches per sample by the number of

261   ovigerous individuals per sample. Total reproductive output was calculated by multiplying

262   average clutch size × the proportion of ovigerous adults per sample × total adult density per

263   sample.

264   Auto-correlation function (ACF) plots indicated significant temporal autocorrelation in

265   our response variables (measured as time series), violating the assumption of independence. To

266   address this, we followed recommendations by (Zuur et al. 2009) to model autocorrelation

267   structure within generalized least squares (nlme package [Pinheiro et al. 2018] in R software).

268   For each response variable (i.e., each generalized least squares model), we utilized the most

269   flexible autocorrelation structure: auto-regressive moving average, ARMA(p, q). We fit this

270   autocorrelation model at the microcosm level (form = time | microcosm) within generalized least

271   squares, obviating the need to model microcosm as a random effect. We further fit ARMA

272   models to individual time series independently of generalized least squares to confirm these

273   models did a good job of capturing *Daphnia* cycling.

274   All generalized least squares models included harvesting treatment, time and a treatment

275   × time interaction as fixed effects. Biomass response variables were log-transformed, and density

276   response variables were square-root transformed prior to model fitting. Our approach to finding

277   an adequate ARMA(p, q) structure was to fit models with 1-7 total parameters (all possible

278   combinations of p and q: 35 different structures) while retaining all fixed effects and select the

279   model with the lowest AIC. When the best-fitting models were similar in AIC values (<2 Δ

280   AIC), we chose the model with the fewest number of combined p, q parameters to maximize

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281 parsimony. We note that according to (Zuur et al. 2009 and references therein), finding an

282 adequate autocorrelation structure is sufficient to account for non-independence of data, with

283 relatively little to be gained from finding a "perfect" fit. Here, the "adequate" p and q structure

284 was the final autocorrelation structure for each response variable that captured most of the

285 variance explained by temporal autocorrelation.

286   Each model was subjected to this same procedure to determine and incorporate the best

287 fit ARMA(p, q) autocorrelation structure. Once an appropriate ARMA(p, q) structure was

288 identified, we tested the overall significance of fixed effects by dropping these terms from

289 models and comparing nested models using likelihood ratio tests. We tested for differences

290 among harvesting treatment levels using least-squares means contrasts (lsmeans package (Lenth

291 2016) in R software).

292   RESULTS

293 *Harvesting treatment efficacy*

294   Stage-specific harvesting treatments were largely successful in removing the targeted

295 *Daphnia* developmental stage throughout the duration of the experiment (Fig. 1A). Though adult

296 harvesting treatments (low-adult, high-adult) did remove some juvenile biomass, this was

297 significantly less than that removed by the high-juvenile harvesting treatment (contrast, pooled

298 adult harvesting treatments vs. high-juvenile: $p < 0.001$; Fig. 1B). As intended, the high-adult

299 harvesting treatment removed approximately twice the adult biomass of the low-adult harvesting

300 treatment (pairwise contrast: $p < 0.001$), while adult biomass removal in the high-juvenile

301 treatment was negligible (Fig 1C). These treatment effects on juvenile and adult biomass

302 removal were consistent throughout the duration of the experiment (likelihood ratio tests of

303 treatment × time interactions: $p > 0.05$; Fig. 1A).

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304   *Reproduction regulation*

305   In support of reproduction-regulation, adult *Daphnia* comprised 75% of total population

306   biomass in the absence of harvesting (i.e., the control) when averaged over the duration of the

307   experiment (Fig. 2A). In contrast, juveniles dominated total density, comprising 63% of all

308   *Daphnia* in the control on average (Fig. 2B).

309   *Juvenile responses*

310   As hypothesized, juveniles exhibited stronger positive (though statistically insignificant)

311   biomass and density responses to mortality compared to adults (biomass: Fig. 2C vs. 2E; density:

312   Fig. 2D vs. Fig. 2F). We detected compensation, i.e., the lack significant differences between

313   harvesting treatments and the control, in both juvenile biomass (contrast, pooled harvesting

314   treatments vs. control: $p = 0.718$; Fig. 2C, Fig. 3A, Fig. 3B) and juvenile density (contrast,

315   pooled harvesting treatments vs. control: $p = 0.612$; Fig. 2D). While the high-adult harvesting

316   treatment increased juvenile biomass by 12% and density by 13% relative to the no-mortality

317   control (Fig. 2C, Fig. 2D), these differences were not statistically significant (biomass, pairwise

318   contrast: $p = 0.258$; density, pairwise contrast: $p = 0.178$). The only significant differences

319   between harvesting treatment levels occurred between high-adult and high-juvenile treatments

320   (biomass, pairwise contrast: $p = 0.009$; density, pairwise contrast: $p = 0.096$), which produced

321   the most positive and most negative juvenile responses to harvesting, respectively (Fig. 2C, Fig.

322   2D). All treatment effects on juvenile biomass and density were consistent throughout the

323   duration of the experiment (likelihood ratio tests of treatment $\times$ time interactions: $p > 0.05$; Fig.

324   3A, Fig. 3B).

325   *Adult responses*

326   In contrast, adult *Daphnia* biomass was significantly reduced by harvesting treatments

327   when compared to the control (contrast, pooled harvesting treatments vs. control: p = 0.005; Fig.

328   2E, Fig. 3C, Fig. 3D). Reductions in adult biomass occurred across all harvesting treatment

329   levels, including the treatment targeting juveniles (pairwise contrasts: p < 0.05). Similar

330   reductions due to harvesting occurred with *Daphnia* density (Fig. 2F), though this effect was not

331   significant (contrast, pooled harvesting treatments vs. control: p = 0.114, Fig. 2B). These

332   treatment effects on adult *Daphnia* biomass and density were consistent throughout the duration

333   of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05, Fig. 3C, Fig.

334   3D).

335   *Total population responses*

336   Because total *Daphnia* biomass was dominated by adults (Fig. 2A), total biomass

337   responses to harvesting mirrored that of adult biomass: total biomass was significantly reduced

338   by harvesting treatments when compared to the control (contrast, pooled harvesting treatments

339   vs. control: p = 0.022, Fig. 2G). Total density was dominated by juveniles (Fig. 2B), and

340   accordingly, showed compensation in response to harvesting treatments (contrast, pooled

341   harvesting treatments vs. control: p = 0.450, Fig. 2E). Treatment effects on total *Daphnia*

342   biomass and density were consistent throughout the duration of the experiment (likelihood ratio

343   tests of treatment × time interactions: p > 0.05, Fig. 3E, Fig. 3F).

344   *Reproductive responses*

345   *Daphnia* responded to harvesting by increasing mean clutch size (*per capita* reproductive

346   output) relative to the control (contrast, pooled harvesting treatments vs. control: p = 0.025), with

347   the high-juvenile harvesting inducing the greatest increase in clutch size (contrast, high-juvenile

348   treatment vs. control: p = 0.027) (Fig. 4A). The proportion of reproductive adults responded

349   similarly to harvesting treatments, though the effect of harvesting was marginal (contrast, pooled

350   harvesting treatments vs. control: $p = 0.0581$; Fig. 4B). These reproductive responses, when

351   multiplied times the number of adults, resulted in compensation in total reproductive output

352   across treatments (contrast, pooled harvesting treatments vs. control: $p = 0.790$; Fig. 4C). These

353   treatment effects on *Daphnia* reproductive responses were consistent throughout the duration of

354   the experiment (likelihood ratio tests of treatment $\times$ time interactions: $p > 0.05$).

355   DISCUSSION

356   While it is generally assumed that extrinsic mortality (e.g., predation or disease) should

357   reduce population density and thus biomass, this prediction derives from models that overlook a

358   fundamental feature of natural populations: developmental variation. New theory incorporating

359   food-dependent development instead predicts biomass increase in response to mortality (i.e.,

360   biomass overcompensation) (Schröder et al. 2014), and biomass overcompensation underlies

361   much of the higher-order, community-level consequences of developmental variation (Roos et al.

362   2008, Huss and Nilsson 2011, de Roos and Persson 2013, Huss et al. 2014). *Daphnia* biomass

363   responses to mortality in our study were largely consistent with those predicted by

364   reproductively-regulated population theory, yet the magnitude of these effects was lower than

365   expected (de Roos et al. 2007, Schröder et al. 2009). Specifically, juvenile *Daphnia* exhibited

366   compensatory, but not over-compensatory, biomass responses to adult mortality (see also

367   Nilsson et al. 2010). Thus, our work demonstrates that ontogenetic stage-structure mediates

368   population response to extrinsic mortality, but cautions that further research is necessary to

369   illuminate the factors underlying the occurrence of biomass overcompensation across natural

370   populations.

371   *Testing theory: The juvenile response*

372   Our study explored biomass overcompensation within reproductively regulated *Daphnia*

373   populations, which allowed us to test clear hypotheses regarding the importance of food-

374   dependent development in mediating population response to mortality. Theory predicts that when

375   populations are regulated by reproduction, adult mortality should enhance total reproduction and

376   thus juvenile biomass because it reduces competition among adults. In line with this prediction,

377   we found that juvenile *Daphnia*, the non-regulatory stage, exhibited the greatest compensation in

378   biomass in response to mortality, with the strongest response induced by mortality of regulatory

379   adults. As a consequence of compensation in juvenile biomass, mortality elicited a shift in the

380   stage structure of *Daphnia* populations: The percentage of total biomass made up by juveniles

381   increased from 25% (no extrinsic mortality control) to 32% (high adult harvesting treatment),

382   and the percentage of juvenile individuals increased from 63% (no extrinsic mortality control) to

383   71% (high adult harvesting treatment). All of these juvenile biomass and density responses were

384   at least partially driven by a positive effect of mortality treatments on *per capita* adult *Daphnia*

385   clutch size, leading to compensation in total reproductive output across treatments. Thus, we see

386   broad correspondence between compensation in adult reproductive output and compensation in

387   juvenile biomass and density in our study, though our analysis failed to detect differences in

388   reproductive responses among mortality treatment levels.

389   While experiments inducing different types stage-specific mortality and measuring

390   biomass responses are still exceedingly rare, our findings are consistent with other data from

391   reproductively-regulated populations (Schröder et al. 2009, 2014, Nilsson et al. 2010). More

392   specifically, the patterns we recovered regarding different types of stage-specific mortality match

393   the most complete biomass overcompensation test to date on reproductively regulated poeciliid

394   fishes (Schröder et al. 2009). Yet our findings differ from previous work in one main way: these

395 studies detected stage-specific biomass overcompensation in juveniles (Schröder et al. 2009,

396 2014), while we detected biomass compensation (see also Nilsson et al. 2010). Notably, we

397 detected juvenile biomass compensation across all mortality treatments, even when mortality was

398 induced in the juvenile stage. We note that while biomass overcompensation in our experiment

399 was statistically insignificant, high-level adult *Daphnia* harvesting did increase juvenile biomass

400 by 12% and juvenile density by 13% relative to the no-mortality control. Regardless, while our

401 experiment recovered patterns consistent with reproductively regulated population theory, we

402 conclude that our effects are somewhat weak.

403   Several explanations exist for the lack of significant biomass overcompensation. First,

404 *Daphnia* populations undergo multiple types of stage-driven population cycles, the physiological

405 mechanisms behind which remain debated (McCauley et al. 2008, Martin et al. 2013, van der

406 Meer 2016). Intrinsic *Daphnia* population cycles were clearly observed in our study, and most

407 apparent in times series of the adult stage. While our analysis approach allowed us to account for

408 intrinsic *Daphnia* cycling at the microcosm-level, such strong and complex temporal

409 autocorrelation within *Daphnia* population dynamics could still obscure treatment effects.

410 Second, while adult *Daphnia* in our study were reproductively mature, they could also continue

411 to grow (see also Schröder et al. 2009). Thus adult *Daphnia* energy could be allocated to growth

412 rather than reproduction, reducing the magnitude of any positive reproductive response

413 (Ohlberger et al. 2011). However, we compared detailed *Daphnia* size distributions across

414 treatment levels and found no evidence for more subtle shifts in size structure (e.g., within

415 juvenile and adult stages; Supplementary Fig. 2). Third, it is possible that *Daphnia* do not exhibit

416 overcompensation, or do so only under limited conditions. While earlier studies suggested

417 biomass overcompensation in cladocerans (Slobodkin and Richman 1956, Edley and Law 1988),

418   a more recent study (Nilsson et al. 2010) instead showed juvenile biomass compensation in

419   response to stage-independent (i.e., random) mortality. Lack of overcompensation in this study

420   (Nilsson et al. 2010) could have occurred due to: (1) stage-independent mortality, which is

421   expected to elicit weaker effects than stage-specific mortality (de Roos et al. 2007); or (2) the use

422   of a single high mortality level that overwhelmed a potential overcompensatory response. We

423   designed our experiment to include both low and high levels of stage-specific adult mortality in

424   hopes of capturing a positive biomass response, and yet failed to do so. We did however find that

425   juvenile biomass was 16% greater at the high versus low adult mortality level (though this effect

426   was statistically insignificant), suggesting that an even higher mortality level could have

427   captured a potential biomass response peak.

428   Still, both Nilsson et al. (2010) and the present study failed to demonstrate biomass

429   overcompensation in *Daphnia*, raising the question of whether overcompensatory responses only

430   exist within a limited parameter space (e.g., range of mortality levels) for certain taxa. Because

431   overcompensation induces cascading effects of developmental variation on whole communities

432   (de Roos and Persson 2013), weak effects might limit the importance of these responses for

433   natural population dynamics and species interaction webs. Indeed, biomass overcompensation is

434   the necessary prerequisite for several population and community effects of ontogenetic

435   development (Roos et al. 2008, Huss and Nilsson 2011, de Roos and Persson 2013, Huss et al.

436   2014), including emergent facilitation between predators (Roos et al. 2008, Huss et al. 2014) and

437   alternative stable community states (van de Wolfshaar et al. 2006, Gårdmark et al. 2015,

438   Toscano et al. 2016). Clearly, more empirical studies testing for biomass overcompensation in

439   response to stage-specific mortality are needed before biomass overcompensation is presumed a

440   general phenomenon in nature. Understanding the empirical factors that limit the magnitude of

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441   biomass overcompensation in natural systems and incorporating these factors into theory is a

442   major research priority moving forward.

443   *Testing theory: adult response*

444   Consistent with other experiments on reproductively-regulated populations (Schröder et

445   al. 2009, Nilsson et al. 2010), adult biomass declined with mortality in our study. Thus, biomass

446   compensation in our study was a stage-specific phenomenon exhibited only by juveniles. Still,

447   we detected an interesting lack of treatment effects on adult biomass. From theory, we expected

448   that: (1) high adult harvesting would reduce adult biomass the most; (2) high juvenile harvesting

449   would reduce adult biomass the least, and; (3) low adult harvesting would have an intermediate

450   effect on adult biomass. While our low adult harvesting treatment was successful in removing

451   exactly half the adult biomass of the high adult harvesting treatment, we found no difference in

452   long-term adult biomass between these treatments. Furthermore, harvesting juveniles produced

453   the same reduction in adult *Daphnia* biomass, despite the juvenile mortality treatment being

454   extremely accurate in capturing juveniles and not adults. One potential contributing factor to

455   these results is that our adult harvesting treatments also removed some juvenile biomass.

456   Regardless, these findings show that very different types of stage-specific mortality can produce

457   a similar reduction in adult biomass. Thus, while juvenile biomass responses in our study were

458   largely in line with reproductive regulation (de Roos et al. 2007, Schröder et al. 2009), the

459   mechanisms underlying observed adult biomass responses require further study.

460   *Relevance for natural dynamics*

461   Our findings suggest that *Daphnia*, a key primary consumer within freshwater systems,

462   exhibit remarkable resistance to extrinsic mortality. Despite inducing substantial stage-specific

463   mortality (instantaneous mortality rate in high harvesting level treatments: 0.07 per day, or 50%

464   of stage-specific biomass removed per week), we found compensation in total *Daphnia*

465   population density, suggesting that the increase in juveniles was roughly equal to the loss of

466   adults through harvesting. While total *Daphnia* biomass was reduced by mortality due to the loss

467   of large adults, juvenile biomass compensation suggests that size-selective predators feeding on

468   adult *Daphnia* can help support predators feeding on juveniles (Brooks and Dodson 1965, Huss

469   and Nilsson 2011). Testing this prediction under natural conditions could provide new insight

470   into the role of zooplankton in supporting diverse predator guilds, and more broadly how

471   ontogenetic development mediates energy flow through food webs (Reichstein et al. 2015).

472   *Conclusions*

473   Unstructured theory assuming individual equivalence has long dominated our

474   understanding of population dynamics, yet ontogenetic variation is widespread and structured

475   theory predicts fundamentally different responses to perturbation (de Roos and Persson 2013,

476   Persson and de Roos 2013). At the most basic level, positive biomass responses arise due to the

477   size-scaling of biomass production (e.g., via rates of food consumption, metabolism or mortality)

478   (de Roos et al. 2007 Peters 1983, Werner and Gilliam 1984). Thus while our study used

479   mortality to shift the adult-juvenile competitive balance, our findings extend to any type of

480   perturbation that modifies ontogenetic asymmetry within populations (Schröder et al. 2014).

481   Because such responses have clear applied relevance to the management of exploited populations

482   (de Roos and Persson 2002, Gårdmark et al. 2015), as well as major implications for species

483   interactions and community dynamics (Roos et al. 2008, Huss and Nilsson 2011, de Roos and

484   Persson 2013, Huss et al. 2014), future experiments inducing a range of stage-specific mortality

485   and measuring biomass responses are crucial. Such work could help to determine whether

486 biomass overcompensation as a result of food-dependent development should be incorporated as

487 a core (i.e., default) feature of population and community theory moving forward.

488   LITERATURE CITED

489 Abrams, P. A. 2009. When does greater mortality increase population size? The long history and
490   diverse mechanisms underlying the hydra effect. - Ecol. Lett. 12: 462–474.

491 Boersma, M. et al. 1996. Seasonal patterns in the mortality of Daphnia species in a shallow lake.
492   - Can. J. Fish. Aquat. Sci. 53: 18–28.

493 Brooks, J. L. and Dodson, S. I. 1965. Predation, body size, and composition of plankton. -
494   Science 150: 28–35.

495 de Roos, A. M. and Persson, L. 2002. Size-dependent life-history traits promote catastrophic
496   collapses of top predators. - Proc. Natl. Acad. Sci. 99: 12907–12912.

497 de Roos, A. M. and Persson, L. 2013. Population and Community Ecology of Ontogenetic
498   Development. - Princeton University Press.

499 de Roos, A. M. et al. 2003. The influence of size-dependent life-history traits on the structure
500   and dynamics of populations and communities. - Ecol. Lett. 6: 473–487.

501 de Roos, A. M. et al. 2007. Food-Dependent Growth Leads to Overcompensation in Stage-
502   Specific Biomass When Mortality Increases: The Influence of Maturation versus
503   Reproduction Regulation (WC Wilson and DL DeAngelis, Eds.). - Am. Nat. 170: E59–
504   E76.

505 de Roos, A. M. et al. 2008. Simplifying a physiologically structured population model to a stage-
506   structured biomass model. - Theor. Popul. Biol. 73: 47–62.

507 Edley, M. T. and Law, R. 1988. Evolution of life histories and yields in experimental populations
508   of Daphnia magna. - Biol. J. Linn. Soc. 34: 309–326.

509 Gårdmark, A. et al. 2015. Regime shifts in exploited marine food webs: detecting mechanisms
510   underlying alternative stable states using size-structured community dynamics theory. -
511   Phil Trans R Soc B 370: 20130262.

512 Gurney, W. S. C. et al. 1990. The Physiological Ecology of Daphnia: A Dynamic Model of
513   Growth and Reproduction. - Ecology 71: 716–732.

514 Huss, M. and Nilsson, K. A. 2011. Experimental evidence for emergent facilitation: promoting
515   the existence of an invertebrate predator by killing its prey. - J. Anim. Ecol. 80: 615–621.

516 Huss, M. et al. 2013. Cohort Dynamics Give Rise to Alternative Stable Community States. - Am.
517   Nat. 182: 374–392.

518 Huss, M. et al. 2014. Facilitation of fisheries by natural predators depends on life history of
519   shared prey. - Oikos 123: 1071–1080.

520 Leeuwen, A. van et al. 2014. Ontogenetic specialism in predators with multiple niche shifts
521   prevents predator population recovery and establishment. - Ecology 95: 2409–2422.

522 Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. - J. Stat. Softw. 69: 1–33.

523 Martin, B. T. et al. 2013. Predicting population dynamics from the properties of individuals: a
524   cross-level test of dynamic energy budget theory. - Am. Nat. 181: 506–519.

525 McCauley, E. et al. 1990a. Growth, Reproduction, and Mortality of Daphnia pulex Leydig: Life
526   at Low Food. - Funct. Ecol. 4: 505–514.

527 McCauley, E. et al. 1990b. The Physiological Ecology of Daphnia: Development of a Model of
528   Growth and Reproduction. - Ecology 71: 703–715.

529 McCauley, E. et al. 2008. Small-amplitude cycles emerge from stage-structured interactions in
530   Daphnia–algal systems. - Nature 455: 1240–1243.

531 Miller, T. E. X. and Rudolf, V. H. W. 2011. Thinking inside the box: community-level
532   consequences of stage-structured populations. - Trends Ecol. Evol. 26: 457–466.

533 Nicholson, A. J. 1954. An outline of the dynamics of animal populations. - Aust. J. Zool. 2: 9–
534   65.

535 Nicholson, A. J. 1957. The self-adjustment of populations to change. - Cold Spring Harb. Symp.
536   Quant. Biol. 22: 153–173.

537 Nilsson, K. A. et al. 2010. Complete compensation in Daphnia fecundity and stage-specific
538   biomass in response to size-independent mortality. - J. Anim. Ecol. 79: 871–878.

539 Ohlberger, J. et al. 2011. Stage-specific biomass overcompensation by juveniles in response to
540   increased adult mortality in a wild fish population. - Ecology 92: 2175–2182.

541 Ohlberger, J. et al. 2012. Community-Level Consequences of Cannibalism. - Am. Nat. 180: 791–
542   801.

543 Persson, L. and de Roos, A. M. 2006. Food-dependent individual growth and population
544   dynamics in fishes*. - J. Fish Biol. 69: 1–20.

545 Persson, L. and de Roos, A. M. 2013. Symmetry breaking in ecological systems through
546   different energy efficiencies of juveniles and adults. - Ecology 94: 1487–1498.

547 Pinheiro, J. et al. 2018. R Core Team (2018). nlme: Linear and nonlinear mixed effects models.
548   R package version 3.1-137.

549 Reichstein, B. et al. 2015. Ontogenetic asymmetry modulates population biomass production and
550   response to harvest. - Nat. Commun. 6: 6441.

551 Roos, A. M. D. et al. 2008. Stage-specific predator species help each other to persist while
552   competing for a single prey. - Proc. Natl. Acad. Sci. 105: 13930–13935.

553 Rudolf, V. H. W. and Lafferty, K. D. 2011. Stage structure alters how complexity affects
554   stability of ecological networks. - Ecol. Lett. 14: 75–79.

555 Sarnelle, O. 1993. Herbivore Effects on Phytoplankton Succession in a Eutrophic Lake. - Ecol.
556   Monogr. 63: 129–149.

557 Schröder, A. et al. 2009. Culling experiments demonstrate size-class specific biomass increases
558   with mortality. - Proc. Natl. Acad. Sci. 106: 2671–2676.

559 Schröder, A. et al. 2014. When less is more: positive population-level effects of mortality. -
560   Trends Ecol. Evol. 29: 614–624.

561 Slobodkin, L. B. and Richman, S. 1956. The Effect of Removal of Fixed Percentages of the
562   Newborn on Size and Variability in Populations of Daphnia pulicaria (Forbes). - Limnol.
563   Oceanogr. 1: 209–237.

564 Stibor, H. 1992. Predator Induced Life-History Shifts in a Freshwater Cladoceran. - Oecologia
565   92: 162–165.

566 Toscano, B. J. et al. 2016. Deadly competition and life-saving predation: the potential for
567   alternative stable states in a stage-structured predator–prey system. - Proc. R. Soc. B Biol.
568   Sci. 283: 20161546.

569 Toscano, B. J. et al. 2017. Cannibalism and intraguild predation community dynamics:
570   coexistence, competitive exclusion, and the loss of alternative stable states. - Am. Nat.
571   190: 617–630.

572 Turchin, P. 2003. Complex Population Dynamics: A Theoretical/empirical Synthesis. - Princeton
573   University Press.

574 van de Wolfshaar, K. E. et al. 2006. Size-Dependent Interactions Inhibit Coexistence in
575   Intraguild Predation Systems with Life-History Omnivory. - Am. Nat. 168: 62–75.

576 van der Meer, J. 2016. A paradox in individual-based models of populations. - Conserv. Physiol.
577   in press.

578 Wagner, A. et al. 2004. Initiation of the midsummer decline of Daphnia as related to predation,
579   non-consumptive mortality and recruitment: a balance. - Arch. Für Hydrobiol. 160: 1–23.

580 Werner, E. E. and Gilliam, J. F. 1984. The ontogenetic niche and species interactions in size-
581   structured populations. - Annu. Rev. Ecol. Syst.: 393.

582   Wyngaard, G. A. and Chinnappa, C. C. 1982. General biology and cytology of cyclopoids. - In:
583   Harrison, F. W. and Cowden, R. R. (eds), Developmental biology of freshwater
584   invertebrates. Liss, pp. 485–533.

585   Yang, L. H. et al. 2008. What Can We Learn from Resource Pulses. - Ecology 89: 621–634.

586   Zuur, A. et al. 2009. Mixed Effects Models and Extensions in Ecology with R. - Springer
587   Science & Business Media.

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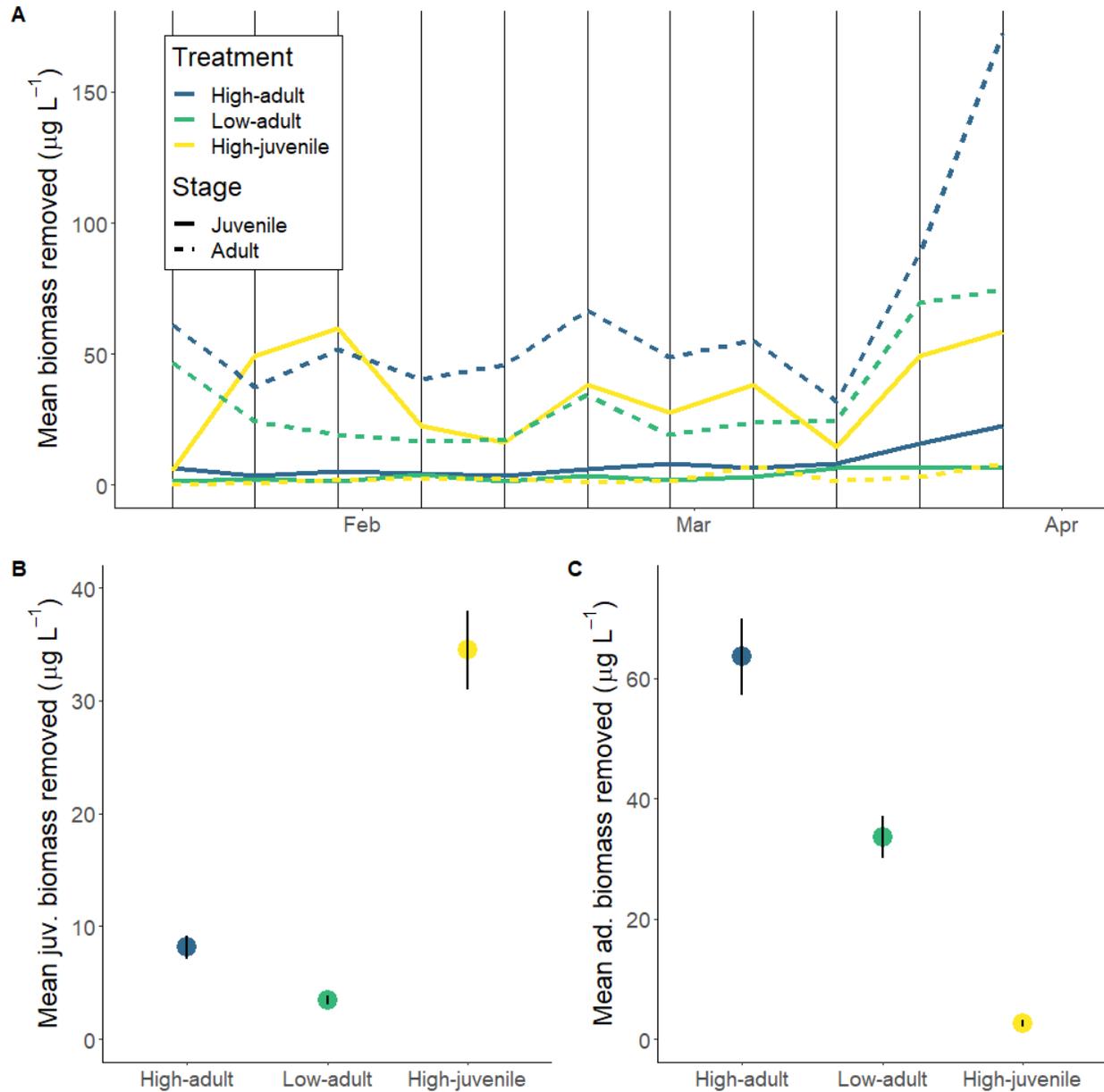
Fig. 1. *Daphnia* biomass removed by harvesting treatments. (A) Time-series of mean juvenile and adult biomass removed (averaged across treatment replicates) over the duration of the experiment. Vertical black lines indicate the days on which *Daphnia* were harvested. (B) Mean juvenile biomass removed per harvesting event ± 1 standard error over the duration of the experiment (n = 11 harvesting events total). (C) Mean adult biomass removed per harvesting event ± 1 standard error over the duration of the experiment.
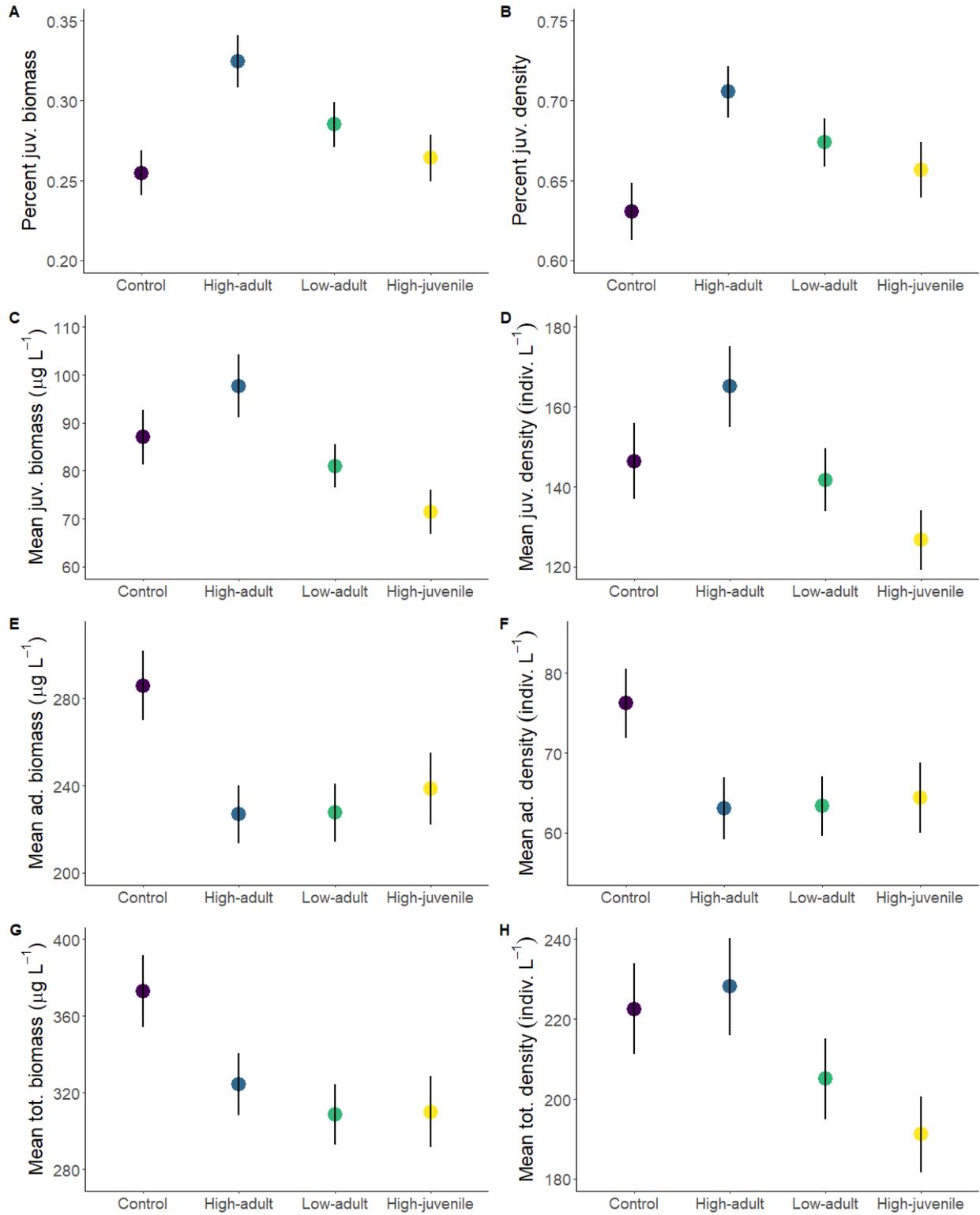
598
599   **Fig. 2.** *Daphnia* population metrics (mean ± 1 standard error) averaged over the duration of the

600   experiment for control and harvesting treatments. (A) Mean percent juvenile biomass. (B) Mean

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601   percent juvenile density. (C) Mean juvenile biomass. (D) Mean juvenile density. (E) Mean adult

602   biomass. (F) Mean adult density. (G) Mean total biomass. (H) Mean total density.
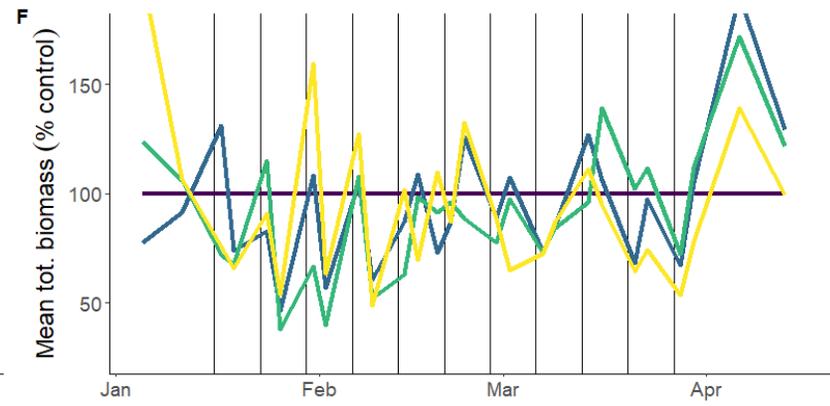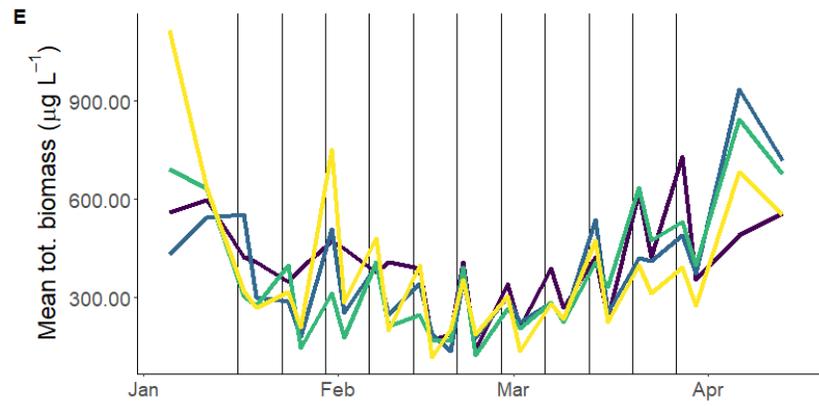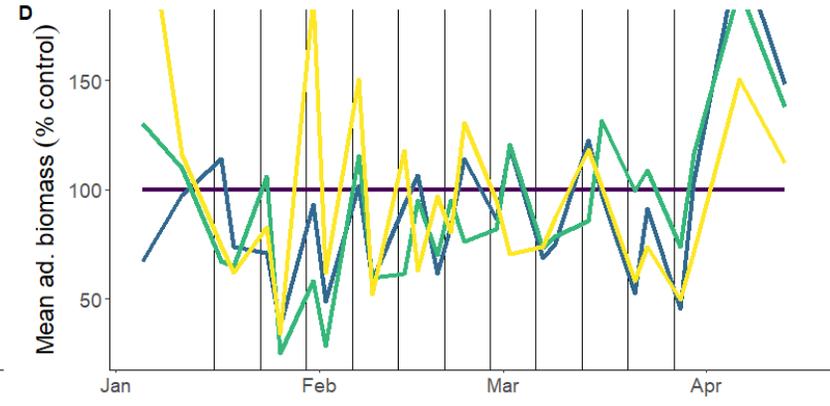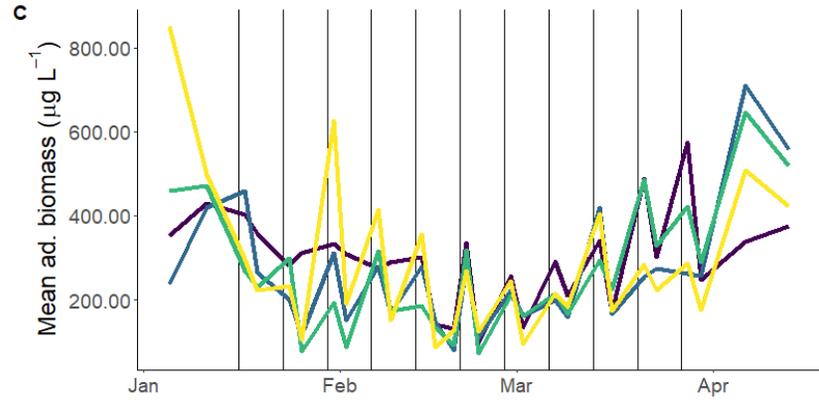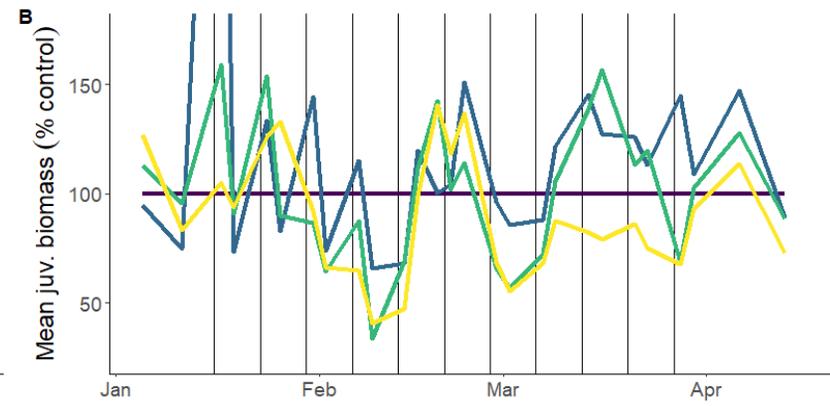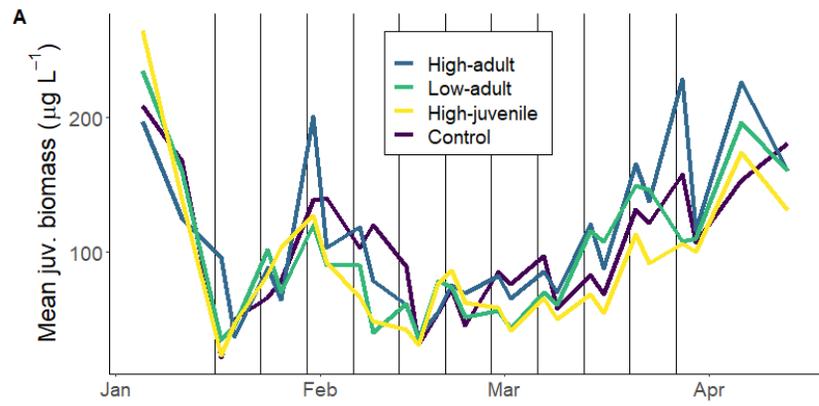
Fig. 3. Time-series of mean *Daphnia* biomass for control and harvesting treatments (A, C, E) and mean *Daphnia* biomass normalized as percent of control biomass (B, D, F) over the duration of the experiment. Vertical black lines indicate the days on which *Daphnia* were harvested. Error bars omitted for clarity.
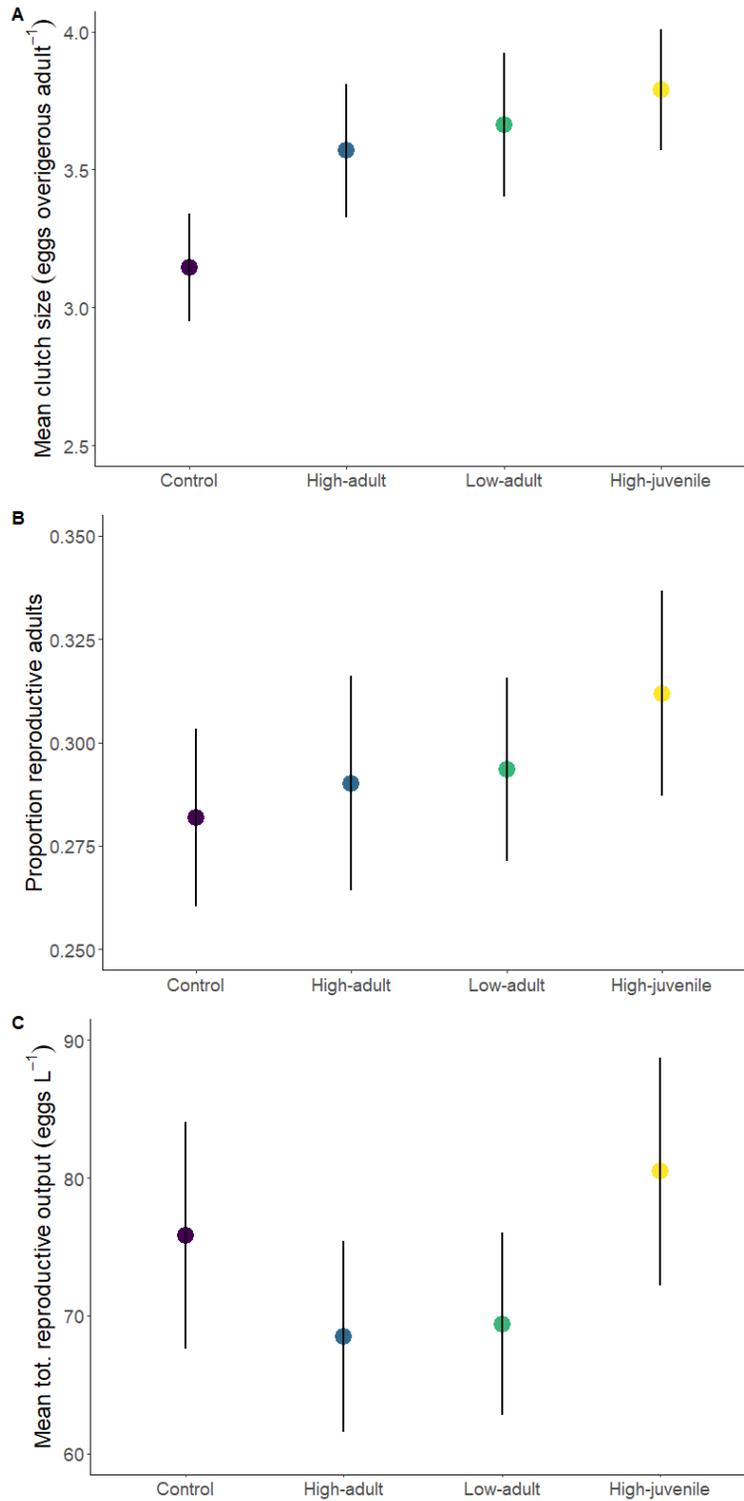
Fig. 4. *Daphnia* reproductive responses to harvesting treatments averaged over the duration of the experiment. (A) Mean clutch size ± 1 standard error (a *per capita* measure). (B) Mean

proportion of reproductive (ovigerous) adults ± 1 standard error (C) Mean total reproductive

output ± 1 standard error (a population-level measure). Total reproductive output was calculated

by multiplying mean clutch size × the proportion of ovigerous adults per sample × total adult

density per sample.