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11 Mummichog, *Fundulus heteroclitus*, Responses to Long-Term, Whole-Ecosystem Nutrient
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45   **Abstract**

46   The effects of eutrophication on coastal plants and sessile animals are becoming well known,

47   but responses of mobile species are less well studied. Here, we link variation in abundance,

48   biomass, body size, growth rate, and resource utilization in mummichogs (*Fundulus*

49   *heteroclitus*) > 40 mm in length to experimental nutrient enrichment in Plum Island Sound,

50   Massachusetts, USA. To mimic cultural eutrophication, dissolved fertilizer was released into

51   replicate saltmarsh creeks on each rising tide throughout entire growing seasons. In the

52   summer of the 6th year of enrichment, we released coded-wire tagged mummichogs into

53   nutrient-enriched (n = 3733 fish) and reference (n = 3894 fish) creeks and recaptured them over

54   the next two months. We found increased abundance (by 37%), biomass (58%), body size (8%),

55   and herbivory (115%, measured as photosynthetic gut pigment content) in nutrient-enriched

56   creeks, although body condition was unaffected. However, individual growth rates were 43%

57   lower in nutrient-enriched creeks. Nutrient enrichment stimulated primary production causing

58   a bottom-up enrichment of the food web, which fostered increased biomass and body size.

59   However, the reduction in growth rate indicates an adverse consequence of long-term nutrient

60   enrichment. This negative effect occurred in the absence of increased hypoxia in these highly

61   tidally (4-m amplitude) flushed study creeks. The mummichog is an important predator/grazer

62   in salt marshes, and nutrient-induced alterations in biomass or resource utilization will directly

63   or indirectly affect lower trophic levels, including benthic algae, thereby impacting the

64   ecosystem-wide response to eutrophication.

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Introduction

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68　　　An accelerated global nitrogen cycle has more than doubled the flow of nitrogen from

69　terrestrial to coastal marine ecosystems (Vitousek et al. 1997, Galloway et al. 2008, Canfield et

70　al. 2010), contributing to increased nutrient loads in estuaries (Deegan et al. 2012). As a result,

71　cultural eutrophication has become widespread, and nearly 70% of North American estuaries

72　have been classified as moderately to highly eutrophic (NOAA 1999). Eutrophication leads to

73　degraded environmental conditions including the loss of submerged vegetation, harmful algal

74　blooms, changes in primary producer communities, dietary changes in consumers, habitat loss,

75　excess carbon production, and oxygen depletion (Deegan 2002, Valiela et al. 2004, Powers et al.

76　2005, Diaz and Rosenberg, 2008, Conley et al. 2009). Estuarine salt marshes provide important

77　ecological and economic services including nutrient removal, storm protection for coastal cities,

78　carbon sequestration, and habitat/provisioning for numerous species of fish, birds and

79　invertebrates (Barbier et al. 2011). Many of the services that may be disrupted or altered by

80　eutrophication support higher trophic levels (e.g., including mobile species associated with

81　fisheries).

82　　　Many studies detail the responses of plant and benthic communities to eutrophication,

83　and effects on abundance, community structure, species composition, diet, and growth

84　allocation are becoming well documented (Khan and Ansari 2005, Darby and Turner 2008,

85　Fleeger et al. 2008, McFarlin et al. 2008, Turner et al. 2009, Gedan et al. 2011, Johnson 2011,

86　Fox et al. 2012). However, less is known about the responses of nekton (Breitburg 1992,

87　Deegan 2002, Essington and Paulson 2010). Eutrophication may have highly disparate effects

88　on nekton. On one hand, the abundance or production of nekton may be reduced by nutrient-

89   enrichment effects on habitat quality or by oxygen depletion (Eby et al. 2005, Valiela et al.

90   2004). On the other, increases in nekton abundance from elevated primary production and

91   stimulated food webs may accompany eutrophication in the absence of hypoxia (Nixon and

92   Buckley 2002, Breitburg et al. 2009).

93   Although nutrient enrichment may increase nekton abundance in the short term, long-

94   term ecological impacts, especially in the absence of hypoxia, are poorly understood. For

95   example, increased abundance associated with eutrophication may lead to indirect effects

96   through density-dependent increases in intraspecific competition (Rose et al. 2001). Weisberg

97   and Lotrich (1986) showed that, with no increase in food availability, a doubling of population

98   density in mummichog (*Fundulus heteroclitus*) led to a 50% decrease in growth rate and that a 4

99   x increase in population size led to increased mortality, indicating that increased abundance

100   may initiate cascading effects on populations. However, nutrient enrichment may also alter

101   food resources, and resource utilization and population responses should therefore both be

102   examined in order to elucidate the mechanisms by which nekton respond to eutrophication.

103   Mummichog is a small killifish (15 to ~ 120 mm TL) that comprises a high proportion of

104   the numbers and biomass of nekton communities in US Atlantic coast salt marshes (Kneib and

105   Stiven 1982, Kneib 1986, Allen et al. 1994, Deegan et al. 2007). Mummichogs are opportunistic

106   omnivores. Juveniles feed on plant material, benthic microalgae, detritus, and small benthic

107   invertebrates, while adults (~ 40 mm and greater in total length, TL) consume macroalgae, grass

108   shrimp (*Palaemonetes pugio*), amphipods, snails, and smaller fish (including juvenile

109   mummichogs) (Kneib and Stiven 1978, Allen et al. 1994, Currin et al. 2003). Mummichogs

110   contribute to top-down regulation of benthic algal and benthic invertebrate communities and

111   may have profound effects on ecosystem structure and function (Kneib 1986, Allen et al. 1994,

112   Sardá et al. 1998, Deegan et al. 2007; Johnson et al. 2009). Furthermore, the mummichog is

113   considered a sentinel of environmental health in estuaries (Weis et al. 2001, Finley et al. 2009).

114   We report here the results of an ecosystem-scale manipulation experiment and a tag

115   and recapture study of mummichogs conducted in tidal creeks experiencing long-term nutrient

116   enrichment. To rigorously quantify responses to nutrient enrichment at single point in time,

117   estimates of abundance, body size, biomass, individual growth rate, resource utilization (i.e.,

118   herbivory), and body condition were made over a two month period in the summer of the sixth

119   year of nutrient enrichment.

120   **Methods**

121   **Study Site and Nutrient Enrichment**

122   Our research is part of the TIDE project (**T**rophic cascades and **I**nteracting control

123   processes in a **D**etritus-based aquatic **E**cosystem) conducted in the Plum Island Estuary (PIE),

124   Massachusetts, USA (Figure 1, 42.73' N, 70.84' W) (Deegan et al. 2007, Deegan et al. 2012).

125   PIE's salt marshes are typical of New England (Niering and Warren 1980), and are characterized

126   by ubiquitous 3-4 m wide bands of *Spartina alterniflora* along the creek-low marsh edge and

127   mosquito ditches. The high marsh consists of expansive areas of *S. patens* and permanently

128   flooded saltmarsh ponds. Tidal amplitudes reach ~4 m during spring and ~2-3 m during neap

129   tides, and all creek water drains into Plum Island Sound at low tide. Mummichogs have access

130   to the flooded marsh on spring high tides, and move locally between interconnected creeks,

131   ditches, ponds, and small isolated pools that retain creek water (Sweeney et al. 1998, Corman

132   and Roman 2011, Able et al. 2012).

133   Four first-order saltmarsh creeks were selected because of their similarities in physical

134   characteristics (e.g., size, substrate, tidal amplitude, and physiognomy, Table 1), vegetation,

135   fauna, and plant and animal zonation (Johnson et al. 2007). Since 2004, two of these creeks

136   have been nutrient enriched daily during entire growing seasons (from ~May 1st – September

137   15th) at target concentrations in flooding creek water of 50-70 μmol $NO_3^- L^{-1}$ and 4 μmol $PO_4^{3-} L^{-1}$

138   (Deegan et al. 2007). These concentrations are consistent with those in estuaries experiencing

139   moderate to high eutrophication (NOAA 1999, EPA 2002, Deegan et al. 2012). The other two

140   creeks have served as unmanipulated references (Figure 2) with background concentrations of

141   ~3.5 μmol NO_3^- and ~0.9 μmol PO_4^{3-} / L (Deegan et al. 2007). No change in the patterns of

142   dissolved oxygen concentration has been observed in TIDE reference or nutrient-enriched

143   creeks over the course of nutrient manipulation (Deegan, unpublished data).

144   **Abundance and Density Estimates**

145   Each nutrient-enriched and reference creek was divided into two reaches of

146   approximately equal length (Figure 2) to examine patterns at a finer spatial scale and to

147   coordinate with routine fish monitoring using flume nets (see below). To estimate abundance,

148   biomass, body size and growth rates, mummichogs were captured, tagged and released daily in

149   each creek reach from July 7 to 17, and then recaptured from July 27 to September 15, 2009.

150   A total of 7828 fish were captured for tagging on ebbing tides using standard minnow

151   traps. The two-piece traps were torpedo-shaped, 49.1 cm in length and 22.9 cm in diameter,

152   with 2.5 cm openings on both ends. The trap's 0.6 mm mesh most efficiently retains

153   mummichogs > 40 mm TL (Kneib and Craig 2001, Deegan unpublished data). No population

154   information was collected during initial capture. After transport to a nearby field laboratory,

155   fish were anesthetized with clove oil, measured (± 1 mm TL), individually marked with

156   sequential coded-wire tags (Northwest Marine Technology, Shaw Island, WA, USA), and held at

157   least 2 h in aerated tanks to allow for recovery. The size range of tagged fish was 31-113 mm TL

158   with a median length of 54 mm (Figure 3). Fish that died during the recovery period were

159   enumerated and removed. Mortality was 2.5% (Lockfield, 2011), and a total of 7627 tagged fish

160   were released; 3733 into nutrient-enriched and 3894 into reference creeks. Tagged fish were

161   released at high tide into the same creek reach where they were captured. After a minimum of

162   10 days from the last release, fish were recaptured and examined in the field for tags by passing

163   fish through a Northwest Marine Technology V-Detector.

164   Marked and unmarked mummichogs were enumerated upon capture in standard

165   minnow traps during the first 2 weeks of the recapture period to estimate abundance in each

166   creek reach using the Lincoln-Peterson method (Seber 1973). From 12-20 minnow traps were

167   placed evenly across each creek reach and fished for varying periods of time on ebbing tides,

168   and each creek reach was sampled every 1-3 days. Marked fish were removed from the

169   population for growth analysis. Non-marked fish were returned to the population. Recapture

170   rates were calculated as the percentage of recaptured fish from the number of tagged fish

171   released into each creek reach. Prior work at our study site (Sweeney et al. 1998) and with

172   coded-wire tags in mummichogs (Able et al. 2006, Kneib 2009) indicate assumptions (a closed

173   population, an equal probability of capture of marked and unmarked fish, equal mortality of

174   marked and unmarked fish, and complete mark retention) were reasonably met. Abundance

175   from each minnow-trap collection was estimated and averaged to generate a creek-reach mean

176   (mummichogs creek reach^{-1}) and 95% confidence interval following Able et al. (2006) and Kneib

177 (2009). However due to a low resampling efficiency in the upper reach of fertilized creek 1 (<

178 5% of the marked fish were recaptured), abundance in only seven creek reaches was estimated

179 (Table 3). High variation in resampling efficiency appears to be common with mummichogs

180 (Kneib 2009), and the investigator travel time to the upper reach of fertilized creek 1 was the

181 greatest among the reaches, reducing available sampling time on each visit. Abundance for

182 each creek (mummichogs creek^{-1}) as a whole was estimated by combining data from both

183 reaches of the same creek.

184 As part of TIDE's long-term, routine monitoring program, flume nets were deployed

185 monthly to estimate the density of mummichogs utilizing the flooded marsh during nighttime

186 spring high tides. Flume nets were built on the high marsh platform at the *S. alterniflora – S.*

187 *patens* interface (i.e., flume nets were placed 2 m from the marsh edge and included *S.*

188 *alterniflora* and *S. patens*). Two replicate flume nets (2 m in width, 5 m in length and with 4.76-

189 mm mesh sides) were fished in each creek reach on one night during the first two weeks of the

190 mummichog recapture period, and data were used to compare with mark-recapture results.

191 Mummichogs were enumerated, measured (± 1 mm TL), and individually weighed (wet weight,

192 ± 0.09 g). Previous research in PIE indicates that flume nets retain juvenile mummichogs ~ 20

193 mm TL and greater (Deegan, unpublished data). Densities (mummichogs m^{-2}) for two size

194 classes (≥ 20 and ≥ 40 mm TL) were calculated for each creek reach (n = 2) by dividing the

195 number of fish trapped of the appropriate size range by the area sampled by the flume net.

196 Mark-recapture abundance (mummichogs creek^{-1}) estimates were converted to density

197 (mummichogs m^{-2}) using the watershed area (defined as the marsh area covered by water

198 during spring high tides) for each creek (Table 1). The watershed area of each creek reach was

199   not known with confidence, and creek-reach density was not calculated by mark-recapture

200   methods (but was estimated from flume net data collected in each reach). Error terms and

201   degrees of freedom in comparisons among creeks were based on creek-level replication (2

202   nutrient-enriched and 2 reference creeks). All error terms throughout the text are standard

203   deviations based on variation between the two replicate creeks unless otherwise noted. t-tests

204   compared fish densities in nutrient-enriched (n = 2) and reference creeks (n = 2) estimated from

205   mark-recapture data and from flume-net data. Independent population estimates in each

206   creek reach were available from the mark and recapture study (as abundance of fish > 40 mm

207   TL in the watershed) and from flume-net samples (as density of fish ≥ 40 mm TL utilizing the

208   flooded marsh) from the same time period. They were compared with Spearman's rank

209   correlation coefficient (n = 7).

210   **Body size, biomass and growth rate**

211   Mummichog length-frequency distributions were derived from tagged fish (Figure 3).

212   Length distributions from nutrient-enriched (n = 3825 fish) and reference (n = 4003 fish) creeks

213   were asymmetric (i.e., skewed to the right), and distributions were compared using a two-

214   sample Kolmogorov-Smirnov test.

215   Biomass (mg dry wt m^{-2}) was also estimated from tagged fish. The length for each fish

216   was converted to wet weight from a length-weight regression (where $\log_{10}$ wet weight =

217   2.874*$\log_{10}$ length - 4.7432, R^2 = 0.8751) determined from measurements of 450 fish collected

218   during the recapture period. A wet to dry conversion factor (0.234) was used to estimate dry

219   weight. Biomass in each creek was determined by multiplying the mean dry weight individual^{-1}

220   in a creek by the density (mummichogs m^{-2}) estimated by mark-recapture for that creek. A t-

221   test was used to compare biomass in nutrient-enriched (n = 2) and reference treatments (n = 2).

222   Individual growth rates were determined on tagged fish captured from throughout the

223   recapture period (333 tagged fish were recovered in nutrient-enriched, and 570 were

224   recovered in reference creeks). Each fish was processed by recording its length and wet weight

225   as above, and the coded-wire tag was recovered from the dorsal musculature and read.

226   Archived data were accessed to retrieve the initial length of each recovered fish. Growth rate

227   was calculated by the equation:

228    $$GR = (L_1 - L_0) / (T_1 - T_0),$$

229   where GR is the growth rate of each individual fish, L_0 is the initial length, L_1 is the recovered

230   length, T_0 is the Julian date marked, and T_1 is the date recovered (Teo and Able 2003, Hagan et

231   al. 2007). Growth rate was analyzed by two methods. A t-test employing fish of all sizes was

232   used to compare average growth rates between nutrient-enriched (n = 2) and reference

233   treatments (n = 2). Data from fish of all sizes were also fitted to a growth model to test for a

234   difference between growth coefficients (k) for all sizes. This method eliminates bias that may

235   be present if fish size differed among the sampling units because older, larger fish grow slower

236   than younger, smaller fish (Teo and Able, 2003). Growth rates in creeks with and without

237   nutrient enrichment were compared using a model selection process; data were fit to the

238   Gompertz, Von Bertalanffy and logistic growth models. All three yielded similar results but the

239   highest goodness of fit was achieved with the Gompertz model. To test for an effect of nutrient

240   treatment, the 95% confidence intervals of the calculated difference of the Gompertz growth

241   coefficients (K_{DIFF}) were analyzed. A difference in growth rate was accepted if the 95%

242 confidence intervals of K_{DIFF} between the nutrient-enriched and reference creeks did not

243 include 0. Finally to more finely examine variation in growth rate associated with variation in

244 body size, individual growth rates were calculated separately for sizes greater than or equal to

245 and less than the median length (54 mm TL) of tagged mummichogs.

246   Linear regression was used to test for density-dependent effects on mummichog growth

247 rate. A standardized growth rate, obtained by dividing the individual growth rate by the length

248 cubed of the same fish, was used to account for age-biased growth. Density of mummichogs ≥

249 40 mm TL from flume-net samples from each creek reach (creek-reach density data were

250 unavailable from the mark-recapture study) was used as the independent variable and mean

251 standardized growth rate from the same creek reach was used as the dependent variable (n=

252 8).

253 **Condition**

254   Fulton's condition factor was also measured on recaptured mummichogs (n = 903).

255 Condition factor was calculated following Nash et al. (2006) and Mustac and Sinovcic (2010):

256 $$K = (W_g / L_{mm}^3) * 100{,}000{,}$$

257 where K is condition, W_g, is the weight in grams, and L_{mm} is the total length in millimeters. A t-

258 test was used to compare condition factor among the nutrient-enriched (n = 2) and reference

259 creeks (n = 2). Linear regression was used to test for density-dependent effects on condition by

260 comparing fish density (mummichogs ≥ 40 mm TL from flume net samples) with mean fish

261 condition among the creek reaches (n= 8).

262 **Herbivory**

263   Photosynthetic gut pigment content was measured as a proxy of herbivory in selected

264   mummichogs collected during the recapture period from nutrient-enriched (n = 48 fish with a

265   range of 42-83 mm TL and a mean TL = 60.0 ± 9.4 mm) and reference creeks (n= 128 with a

266   range of 30-91 TL and a mean TL = 52.8 ± 9.6 mm). Fish collected in standard minnow traps

267   fished for ≤ 30 min on the falling tide were selected for this analysis. Fish were placed on dry

268   ice in the field, and stored at -80°C until processed. Each fish was thawed and the stomach was

269   removed after measuring fish length as above. Stomach contents were rinsed into a 50-ml

270   tube, acetone was added to achieve a 75% acetone:water ratio, and samples were refrigerated

271   overnight (16-20 h). Then after reaching room temperature, tube contents were centrifuged,

272   and the fluid decanted and filtered with 0.22-µm diameter nylon filters. Pigment

273   concentrations were measured using a Turner model 10 flourometer before and after

274   acidification, and values were converted into µg chlorophyll (chl) a fish^{-1} and µg phaeopigment

275   fish^{-1} and then standardized by fish length cubed for statistical analysis. The ratio of chl

276   a:phaeopigment for each fish (Fleeger et al. 1999) was also calculated. t-tests, but without

277   creek-level replication (fish from replicate creeks were pooled because of an unbalanced

278   sample size per creek), were used to compare standardized photosynthetic gut pigment

279   content and the ratio of chlorophyll a:phaeopigment in nutrient-enriched and reference creeks.

280   **Results**

281   One tagged and recaptured mummichog was 39 mm TL at the time of release; all other

282   recaptured fish were > 40 mm at the time of release. Minnow traps used to collect fish for

283   tagging and for recapture rarely captured fish < 40 mm TL (33 out of the 7828 fish tagged were

284   < 40 mm TL). Thus, data derived from the mark-recapture study most effectively assessed

285   abundance and growth rate of mummichogs > 40 mm TL.

286   **Abundance and Density**

287   A total of 903 released fish were recaptured. Recapture rates varied from 8.5 to 17.1%

288   among creek reaches, and overall 12.8% were recaptured (Table 3). About 80% of the fish were

289   recovered in the same reach where released; 16% were recovered in the adjacent reach.

290   Although 3.7% of the mummichogs moved to a nearby study creek, no mummichogs moved

291   between the nutrient-enriched and reference creeks. The mean TL at the time of release for

292   recaptured fish was 56.4 ± 10.7 in reference creeks and 59.0 ± 9.9 mm in nutrient-enriched

293   creeks. Recaptured mummichogs in reference creeks exhibited a mean TL of 59.4 ± 10.2, and

294   recaptured fish in nutrient-enriched creeks had a mean TL of 62.1 ± 9.7 mm. The initial range in

295   TL (i.e., when tagged) of the mummichogs we recaptured was 39-98 mm, and length at the time

296   of recapture ranged from 42-98 mm.

297   Estimates of mummichog abundance were based on successive recaptures derived from

298   mark-recapture data, and means varied from 13,315 to 27,744 mummichogs creek reach^{-1}, ±

299   3,600-8,000 (95% confidence intervals) (Table 3). Mean whole-creek density was 0.59 ± 0.07

300   mummichogs m^{-2} in reference creeks, while fertilized creeks averaged 0.81 ± 0.04 mummichogs

301   m^{-2}. This 37% higher abundance in the nutrient-enriched creeks was marginally significant (DF =

302   2, t = 4.07, p = 0.055).

303   Flume-net derived estimates of the density of mummichogs ≥ 40 mm TL from the high

304   marsh in fertilized creeks averaged 0.35 ± 0.16 compared to 0.15 ± 0.14 mummichogs m^{-2} in

305   reference creeks, but densities did not differ statistically (DF = 2, t = -1.60, p = 0.251). Flume-

306   net derived mean density estimates of mummichogs ≥ 20 mm TL were 0.39 ± 0.47 in reference

307   compared to 0.85 ± 0.39 m^{-2} in fertilized creeks. This difference also was not significant (DF = 2,

308   t = -1.57, p = 0.258). The abundance estimated from the mark-recapture study (mummichogs >

309   40 mm TL creek $reach^{-1}$) and density of mummichogs (mummichogs ≥ 40 mm TL m^{-2}) estimated

310   from flume nets followed similar trends among the creek reaches. These two independent

311   estimates were positively correlated and marginally significant (correlation coefficient = 0.71; p

312   = 0.055).

313   **Body Size, Biomass, and Growth Rate**

314   The Kolmogrov-Smirnov test indicated that mummichog length distributions in tagged

315   fish were significantly different in reference and nutrient-enriched creeks (Figure 3, Ksa = 8.97,

316   p < 0.001). Nutrient-enriched creeks had a higher proportion of larger fish than reference

317   creeks.

318   Mummichog biomass was significantly higher in fertilized creeks (DF = 2, t = 5.47, p =

319   0.031). Biomass in reference creeks averaged 272.72 ± 12.71, and biomass averaged 445.23 ±

320   40.34 mg dw m^{-2} in nutrient-enriched creeks.

321   Across all sizes, mean linear mummichog growth rates (0.103 ± 0.085 mm d^{-1} in

322   reference creeks and 0.072 ± 0.065 mm d^{-1} in fertilized creeks) were significantly faster in

323   reference creeks (DF = 2, t = 6.44, p = 0.023). For mummichogs < 54 mm TL (the median length

324   of tagged fish), growth rate averaged 0.123 in reference and 0.083 mm d^{-1} in nutrient-enriched

325   creeks. For mummichogs ≥ 54 mm TL, the mean growth rate was 0.080 in reference and 0.066

326   mm d^{-1} in nutrient-enriched creeks.

327   Growth rate was inversely related to initial length for recaptured mummichogs (based

328 on linear regression, where growth rate = -0.0025 * initial length + 0.2358, R^2 = 0.1088, p <

329 0.001). Mummichog growth data were therefore fitted to a Gompertz growth model to remove

330 this length-growth rate bias. Reference creeks had a Gompertz growth coefficient of 0.0041 ±

331 0.0003 and fertilized creeks had a growth coefficient of 0.0029 ± 0.0004. The calculated

332 difference, K_{DIFF} (= 0.0012), had 95% asymptotic confidence intervals that did not include 0 (-

333 0.0021, -0.0005); therefore, we concluded that the growth coefficients of the two treatments

334 were different and that faster growth rate occurred in the reference creeks. Finally,

335 standardized growth rate decreased as the density of mummichogs increased among the creek

336 reaches (Figure 4). This relationship was significant (based on linear regression, where

337 standardized growth rate = -0.1149 * density + 0.0875, R^2 = 0.7927, DF =7, p = 0.003).

338 **Condition**

339   Mean condition factor did not differ between reference (1.32 ± 0.23) and nutrient-

340 enriched creeks (1.27 ± 0.13) (DF = 2, t = 1.55, p = 0.262). Furthermore, condition did not vary

341 with mummichog density (based on linear regression, where condition = -0.1738 * density +

342 1.344, R^2 = 0.2345, DF = 7, p = 0.224).

343 **Herbivory**

344   Mean gut pigment content was 5.20 ± 10.30 in reference and 21.91 ± 28.21 µg chl a fish$^-$

345 ^{1} in nutrient-enriched creeks. Standardized chl a gut content (data not shown) differed

346 between nutrient-enriched and reference creeks (t = -3.71, p $\leq$ 0.001). Phaeopigment gut

347 content was consistently lower than chl a in the same fish, and averaged 2.47 ± 5.54 in

348 reference creeks and 4.07 ± 4.79 µg fish^{-1} in nutrient-enriched creeks. The ratio of chl

349   *a*:phaeopigment was significantly higher (t = -2.56, p = 0.014) in nutrient-enriched creeks and

350   averaged 1.23 ± 1.80 in reference and 2.64 ± 3.38 in nutrient-enriched creeks.

351   **DISCUSSION**

352   Mummichog responses in the 6th year of creek-level nutrient enrichment were complex,

353   and positive and negative impacts were both observed. Population biomass and individual

354   body size were higher with nutrient enrichment, although individual growth rates were lower

355   during the two-month summer study period, without change in fish condition. Nutrient

356   enrichment also altered resource utilization as indicated by our proxy (gut photosynthetic

357   pigment content) for herbivory, which indicated a greater use of algal resources in nutrient-

358   enriched creeks. It is unlikely that migration contributed to, or diluted, the observed

359   differences among creeks because mummichogs are well known to exhibit high site fidelity

360   (Sweeney et al. 1998, Hagen et al. 2007, Able et al. 2006). Less than 4% of mummichogs moved

361   from one creek to another creek nearby, and none moved between the reference and nutrient-

362   enriched creeks, a distance of about 1.5 km.

363   The median length of the fish we tagged was 54 mm TL, and recaptured tagged fish

364   ranged in length from 42-98 mm. Previous research on mummichogs indicates this size range

365   includes juveniles (i.e., young-of-the-year) and mummichogs in the second year of life (Teo and

366   Able 2003, Hagan et al. 2007). Although mummichogs are known to experience high summer

367   growth rates (Teo and Able 2003), the growth potential during our recapture period likely

368   differed in the two age classes. On average, juveniles spawned in the spring or early summer

369   should grow faster in the first half of the summer and more slowly in the second half as they

370   reach maturity and begin to reproduce. Second-year fish grow more slowly (Teo and Able

371   2003), and, as adults, likely grow at a more similar rate throughout the growing season.

372   Although the 2-mo study period represents about a third of the growing season and about 10%

373   of the lifespan of mummichog, this length of time has been shown to be effective at identifying

374   differences in growth rate across marsh landscapes (Kneib 2009). Furthermore, routine

375   monitoring by the TIDE project based on length-frequency distributions taken monthly during

376   the summer over several years indicates broadly similar growth rates, ~ 0.1 mm/day, and that

377   growth rate is consistent throughout the summer period for second-year mummichogs (Nelson

378   and Deegan, unpublished). Our research indicated that growth rates were lower in nutrient-

379   enriched creeks compared to reference creeks in both age classes by a similar amount (for fish

380   < 54 mm, mean individual growth in the nutrient-enriched creek was less by 0.0402 mm d^{-1} and

381   for fish ≥ 54 mm, the difference was less by 0.0140 mm d^{-1}). These observations suggest that

382   our methods yielded findings consistent with patterns of growth throughout the growing

383   season in our experimental creeks and across the size range of mummichogs studied. However,

384   it is possible that differences in growth rates associated with nutrient enrichment occurred

385   during or just prior to our study, and thus growth rates may be indicative of the particular

386   period in time during which the study was conducted.

387   The increased individual body size and higher population biomass of mummichogs in the

388   nutrient-enriched creeks can be traced to a bottom-up stimulation of the food web induced by

389   nutrient enrichment. Nitrogen and phosphorous enrichment stimulates benthic algae (Elser

390   2007) which in turn stimulates infaunal and epibenthic invertebrates (Posey et al. 1999, Posey

391   et al. 2002, Johnson 2011, Johnson and Short 2012). Benthic algae and infaunal and epifaunal

392   invertebrates all serve as food resources for mummichogs (Allen et al. 1994). Mummichogs

393   consumed more benthic algae in nutrient-enriched creeks as measured by photosynthetic gut

394   pigment content. Johnson and Fleeger (2009) found that nutrient enrichment led to an

395   increase in the biomass and body size of some infaunal invertebrates, and Johnson (2011) and

396   Johnson and Short (2012) found nutrient enrichment stimulated the abundance and biomass of

397   epifaunal amphipods, isopods, and snails. The mummichog is an opportunistic omnivore and a

398   highly flexible diet should allow it to take advantage of a stimulation of several components of

399   the food web.

400   Individual growth rates, however, revealed that environmental quality at the time of the

401   study was reduced by long-term nutrient enrichment. Mummichog growth rates based on

402   length were significantly lower (by 43%) in fertilized creeks. However, fish condition, based on

403   individual length and weight, was equivalent in all creeks. High condition factor values are

404   generally associated with more robust fish (Nash et al. 2006), and condition values in PIE were

405   similar to or higher than those found in other studies with mummichogs (McMullin et al. 2009),

406   suggesting all experimental creeks provided a high-quality habitat for mummichogs. If bottom-

407   up effects led to an increased quality or supply or food as anticipated, condition would be

408   expected to be higher in the nutrient-enriched creeks. However, the allocation of biomass in

409   fishes to length or body mass varies with environmental conditions such as food supply and

410   season (Rikardsen and Elliott 2000). For example, fish on a low-quality diet may maximize an

411   energy storage strategy over a growth maximization strategy (and thus allocate more growth to

412   body mass over increases in length, Post and Parkinson 2001). Furthermore, life history

413   constraints in mummichogs may dictate a tradeoff between growth in terms of length

414   (increased length may contribute to a reduction in predation) balanced against lipid storage

415   needed to survive winter. Fish in nutrient-enriched creeks may therefore have differed in their

416   absolute growth rate and/or the way they allocated growth. The high condition factor but low

417   growth rate in mummichogs in nutrient-enriched creeks could therefore have been a function

418   of growth allocation. Alternatively, reductions in growth rate may have occurred due to a

419   recent reduction in habitat quality before a change in condition could be manifested. Below,

420   we discuss some of the ways that nutrient enrichment may reduce environmental quality or

421   affect mummichog abundance, diet, or behavior and contribute to reduced or altered growth.

422   These effects took place in the absence of nutrient-induced oxygen depletion in our tidally

423   flushed study creeks.

424   Density-dependent effects via intraspecific competition may have contributed to the

425   lower growth rate in nutrient-enriched creeks. Mummichogs were present in higher

426   abundance and biomass in fertilized creeks which could lead to increased competition for food.

427   Weisberg and Lotrich (1986) found that a doubling of the population density of mummichogs

428   led to a 50% reduction in growth rate due to reductions in the amount of food per fish. Growth

429   rates would slow in the nutrient-enriched creeks if the negative effects of increased

430   competition outweighed the positive effects of nutrient enrichment on resource quantity and

431   quality. The magnitude of higher abundance (37%) and lower growth rate (43%) in nutrient-

432   enriched creeks indicates a density-dependent response similar to that found by Weisberg and

433   Lotrich (1986), and suggests little, if any, net resource benefit to mummichogs was gained from

434   nutrient enrichment at the time of the study. Strong support for the importance of intraspecific

435   competition as a cause of the decreased growth rates comes from our observation that

436   mummichog growth rate decreased significantly with increasing mummichog density among

437   the creek reaches (Figure 4). However, fish condition did not vary with mummichog density as

438   would be predicted if intraspecific competition caused variation in growth rate. A monitoring

439   program with a focus on long-term trends in fish size, biomass and growth is being conducted in

440   TIDE creeks and should improve our understanding the mechanism of and the longer-term

441   nutrient-enrichment effects on mummichogs (Nelson and Deegan unpublished). Our mark-

442   recapture study complements this study in that it takes an intensive and in-depth, but snap-

443   shot look at mummichog responses.

444   The gut contents of mummichogs collected in all creeks contained relatively large

445   amounts of photosynthetic pigment. Visualization-based studies of gut contents, isotope

446   composition studies and density manipulations with mummichogs suggest the source of

447   pigment was benthic algae (Allen et al. 1994, McMahon et al. 2005, Deegan et al. 2007). We

448   also found that gut pigment content increased by 115% in similarly sized fish from nutrient-

449   enriched creeks where mummichogs exhibited a higher chl a:phaeopigment ratio. A nutrient-

450   stimulated increase in algal productivity or an increase in nutritional quality would be expected

451   to increase the availability or attractiveness of algae as a food for mummichogs, leading to

452   increases in herbivory. However if consumption of animal prey was concomitantly reduced

453   with increased herbivory in the nutrient-enriched creeks, the result could be a slower growth

454   rate due to the lower dietary quality/protein content of plant tissue. Such changes in diet and

455   behavior could have contributed to a slower growth rate in the nutrient-enriched creeks.

456   Herbivorous fish have been found to be regulated by the supply of algae in a bottom-up fashion

457   (Flecker et al. 2002), but pathways responsible for food-web stimulation to omnivores are more

458   complex, variable, and difficult to identify (Bruno et al. 2005). Unfortunately available data

459   shed little light on the relative importance of contributions of algal and animal components

460   from the food web to mummichogs, and the variation in those contributions due to nutrient

461   enrichment is also uncertain (e.g., animal contributions to mummichog diet were not examined

462   here). Alternatively, it is possible that the observed increase gut photosynthetic pigment

463   content was due to increased incidental ingestion of algae while foraging for small, cryptic

464   animal prey. In nutrient-enriched creeks increased cover may have been provided by more

465   productive benthic algae increasing incidental ingestion while foraging. However, this seems

466   unlikely because benthic algal biomass was not affected in mudflat or creek-wall habitats in

467   nutrient-enriched creeks in the year our study was conducted, and an increase in marsh-edge,

468   *S. alterniflora* habitat algal biomass (Mitwally and Fleeger 2013) may have been mitigated by

469   nutrient-induced habitat loss (see below). Additional study of nutrient-enrichment effects on

470   resource utilization in mummichogs is needed.

471   Long-term nutrient-enrichment effects on the marsh landscape were accumulating at

472   the time of our study, and resulting habitat degradation may have contributed to a reduction in

473   mummichog growth rate. Deegan et al. (2012) found the marsh-edge, *S. alterniflora* habitat

474   was decreased in TIDE's nutrient-enriched creeks. Nutrient enrichment altered the allocation

475   of biomass in *S. alterniflora* (less was allocated to the sediment-stabilizing roots) and increased

476   microbial decomposition rates of root material, which together decreased soil strength (Deegan

477   et al. 2012). As a result, fractures formed in the soil surface and large sections of the marsh

478   edge collapsed into the adjoining creek channel before our study was conducted. A survey

479   taken at regularly spaced distances along the creek edge in 2009 found that 8.9% of the

480   observations in reference creeks were of an unvegetated muddy substrate (i.e., 91.1% of the

481 bottom was vegetated by *S. alterniflora*), while 24.8% of the nutrient-enriched creeks was

482 unvegetated (Deegan et al. 2012). Many studies (Baltz et al. 1993, Peterson and Turner 1994,

483 Deegan 2002, Minello et al. 2003) have shown the importance of a vegetated creek-marsh edge

484 for saltmarsh nekton, although the high marsh has also been shown to be important in New

485 England mashes for mummichogs (MacKenzie and Dionne 2008). If the *S. alterniflora*-

486 dominated low marsh is an important habitat facilitating increased growth rates or increased

487 feeding opportunities compared to that in the collapsed marsh edge of the nutrient-enriched

488 creeks, or is a significant access point to the high marsh for mummichogs, this observed habitat

489 alteration could contribute to lower growth rates in mummichogs.

490   The mummichog is a high-level grazer and predator in salt marshes (Deegan et al. 2007),

491 and nutrient-induced alterations in biomass or resource utilization may therefore have

492 important repercussions to saltmarsh food webs via direct or indirect effects. For example,

493 Deegan et al. (2007) found that benthic algal biomass was increased by the combination of

494 nutrient enrichment and fish removal in the second year of TIDE fertilization. Our findings

495 indicate that direct herbivory by mummichogs contributed to this interactive effect. The

496 magnitude of eutrophication is often measured by algal abundance or biomass (Ferreira et al.

497 2011). The increase in mummichog biomass coupled with the increase in consumption of

498 benthic algae we observed with nutrient enrichment suggest that mummichogs may slow the

499 rate of increase in algal biomass, and therefore the observed pace of eutrophication in nutrient-

500 enriched creeks. This finding is similar to that of Pascal et al. (2013) for benthic invertebrates in

501 TIDE's nutrient-enriched creeks. However, if negative impacts to fitness occur over the long

502 term affecting populations, mummichog grazing rates may be reduced. This would lead to an

503   accelerated increase in benthic algal biomass, contributing to an increased rate of

504   eutrophication. The abundance, behavior, and growth of infauna, epifauna, and grass shrimp

505   would also vary with changes in mummichog abundance or predation rate (Kneib 1986, Cross

506   and Stiven 1999, Carson and Merchant 2005, Deegan et al. 2007). Therefore, the mummichog

507   should be considered a keystone species that affects the persistence and resilience of saltmarsh

508   ecosystems, with the potential to modify the ecosystem-wide response to eutrophication.

509 **Acknowledgements**

521

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774

775   Table 1. Physical characteristics of study creeks. Creek dimensions and measurements from

776   Johnson et al. (2007). Each creek was divided two similar creek reaches for fish collections.

777

Creek	Total Watershed Area (m^2 x 10^4)	Linear Distance (m)	Distance from Rowley River (km)	Distance from Plum Island Sound (km)	Volume (L x 10^6)	Cross-sectional area (m^2)
Reference 1	6.01	260	1.28	3.74	1.37	5.27
Reference 2	5.32	230	1.43	3.89	2.04	8.89
Fertilized 1	5.91	300	1.31	4.43	3.73	12.42
Fertilized 2	6.59	335	1.31	4.43	3.59	10.73

778

779

780   Table 2. Number of fish tagged and released each day during the mark and recapture study.

781

Date	Nutrient Treatment	Replicate	Reach	# of Fish Released
7/7/09	Reference	1	Upper	523
7/8/09	Reference	1	Lower	277
7/8/09	Reference	2	Upper	615
7/9/09	Reference	1	Lower	558
7/9/09	Reference	2	Lower	503
7/10/09	Fertilized	1	Upper	530
7/10/09	Fertilized	2	Lower	555
7/13/09	Fertilized	1	Lower	518
7/13/09	Fertilized	2	Upper	498
7/14/09	Fertilized	1	Upper	465
7/14/09	Fertilized	2	Lower	266
7/14/09	Fertilized	2	Upper	163
7/15/09	Fertilized	1	Lower	338
7/15/09	Fertilized	2	Upper	400
7/16/09	Reference	1	Upper	467
7/16/09	Reference	1	Lower	256
7/16/09	Reference	2	Lower	400
7/17/09	Reference	2	Upper	295
Total				7627

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784　Table 3. Recapture rates and abundance estimates and 95% confidence intervals of each creek

785　reach derived from mark and recapture study. Not applicable (NA).

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Creek	Replicate	Reach	Recapture rate	Abundance estimate	95% Confidence Interval
Reference	1	Upper	17.1%	13,315	8,576 – 18,054
Reference	1	Lower	17.1%	17,371	12,552 – 22,190
Reference	2	Upper	12.8%	22,069	16,674 – 27,464
Reference	2	Lower	14.6%	14,196	10,569 – 17,823
Fertilized	1	Upper	NA	NA	NA
Fertilized	1	Lower	8.5%	27,113	19,302 – 34,924
Fertilized	2	Upper	11.2%	27,744	21,255 – 34,233
Fertilized	2	Lower	11.7%	25,398	17,006 – 33,790

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790   Figure Legends

791   Figure 1. Aerial photo of the study site, located in the Rowley River drainage which drains into
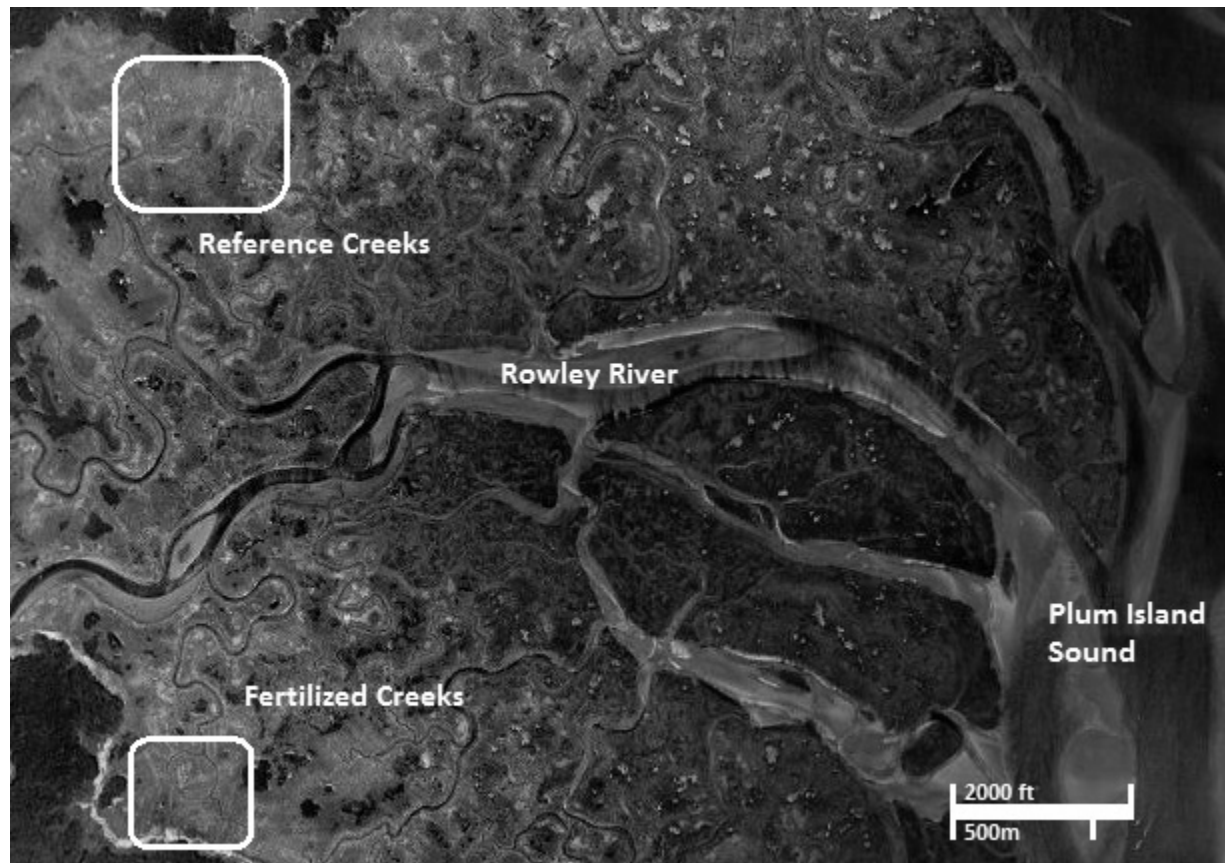792   the Plum Island Sound in northeastern Massachusetts.
793
794   Figure 2. Schematic of TIDE experimental creeks showing fertilizer application point and creek
795   reaches. Each of the creeks is divided into an upper and lower reach. The dripper is the point
796   of the nutrient release.
797
798   Figure 3. Mummichog length-frequency distributions in reference (n = 4003) and nutrient-
799   enriched (n = 3825) creeks of fish tagged for release. Arrows indicate the median length in the
800   reference and nutrient-enriched. Mortality of tagged fish was 2.5% and 3894 tagged fish were
801   released into reference and 3733 tagged fish were released into nutrient-enriched creeks.
802
803   Figure 4. Relationship between mummichog density (mummichogs m^{-2} from flume-net samples)
804   and standardized growth rate. Standardized growth rates are equal to growth rate expressed
805   as mm d^{-1} divided by fish length cubed. The solid line is the best fit linear regression line and
806   the dotted lines are the 95% confidence intervals (n = 8). Symbols are coded to identify specific
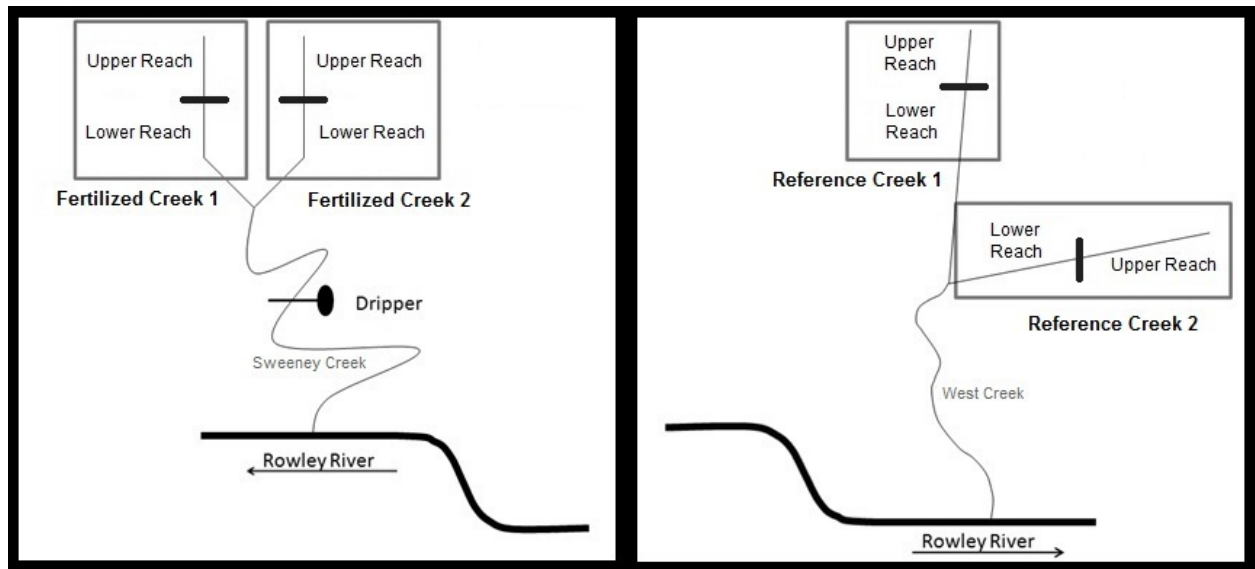807   creek reaches (see Table 3).
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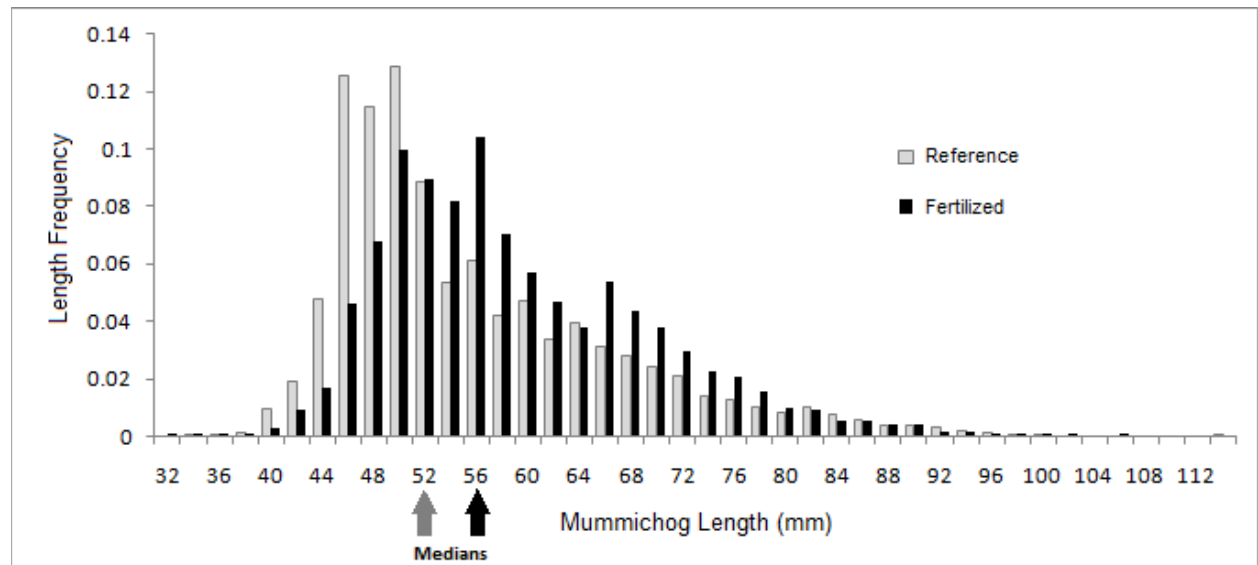Figure 1
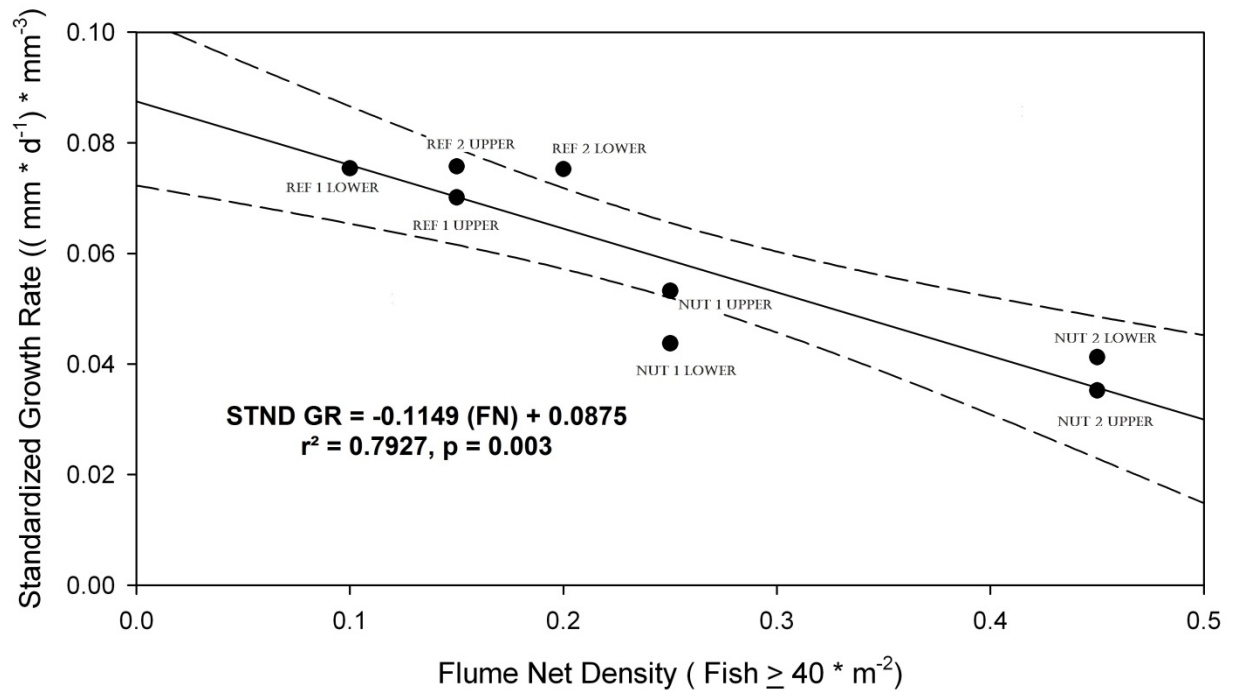
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818   Figure 2
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824   Figure 3
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835   Figure 4