1   Title: Predation drives recurrent convergence of an interspecies mutualism
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50   laboratory studies of fish behaviour were conducted by W.E.F and R.M.B, with assistance from

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Predation drives recurrent convergence of an interspecies mutualism

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ABSTRACT

Mutualisms are important ecological interactions that underpin much of the world's biodiversity. Predation risk has been shown to regulate mutualism dynamics in species-specific case studies; however, we lack studies which investigate whether predation can also explain broader patterns of mutualism evolution. We report that fish-anemone mutualisms have evolved on at least 55 occasions across 16 fish families over the past 60 MY and that adult body size is associated with the ontogenetic stage of anemone mutualisms: larger-bodied species partner with anemones as juveniles, while smaller-bodied species partner with anemones throughout their lives. Field and laboratory studies show that predators target smaller prey, that smaller fishes associate more with anemones, and that these relationships confer protection to small fishes. Our results indicate that predation is likely driving the recurrent convergent evolution of fish-anemone mutualisms and suggest that similar ecological processes may have selected convergence in interspecies interactions in other animal clades.

INTRODUCTION

Mutualistic relationships, in which individuals from two or more species cooperate, are important ecological interactions (Bronstein 1994; Hay *et al.* 2004; Bascompte & Jordano 2007). Partners exchange a variety of resources or services (Grutter 1999; Dixson & Hay 2012; Canestrari *et al.* 2014) and range from opportunistic to obligate in their reliance on one another. Networks of mutualistic interactions can comprise hundreds of species (Thébault & Fontaine 2010) and by mediating competition, they can promote coexistence and increase surrounding biodiversity (Schmitt & Holbrook 2003; Bastolla *et al.* 2009; Weber & Agrawal 2014). External pressures, such as predation, have been shown in a number of case studies to be important for mediating mutualism dynamics: the removal of herbivores resulted in the breakdown of an ant-plant mutualism in Africa (Palmer *et al.* 2008) and the prevalence of nest predation determines whether hosting a cuckoo nestling is detrimental or beneficial in a European host (Canestrari *et al.* 2014). However, the extent to which these kinds of processes can influence convergent patterns of mutualism evolution remains to be formally investigated (Doebeli & Knowlton 1998; Toby Kiers *et al.* 2010; Bittleston *et al.* 2016).

Since first being described off the coast of Borneo in 1868 (Collingwood 1868), the relationships between fishes and anemones have provided tractable models for studying interspecies mutualisms. These partnerships are instigated by the fishes, and partner reliance spans from facultative to obligate for fishes and is facultative for anemones (Fautin 1991). Past research has primarily focused on understanding the ecology and evolution of the 29 obligately associated Amphiprioninae anemonefishes (*Amphiprion* spp. and *Premnas* sp.; *hereafter* 'anemonefishes') (Buston 2003a, 2004; Munday *et al.* 2009) as well as interactions between them and the ten anemone species that they associate with throughout their Indo-Pacific range (Elliott *et al.* 1999; Schmitt & Holbrook 2003; Ollerton *et al.* 2007). While anemones that host

100 fishes gain benefits such as increased access to nutrition or increased aeration (Szczebak *et al.*

101 2013; Lim *et al.* 2016), protection from predators is widely credited as a key benefit that

102 anemonefishes receive through their associations with anemones (reviewed in Fautin 1991).

103 However, given the obligate nature of these relationships, this has proven difficult to

104 conclusively demonstrate. In contrast, while no prior research has investigated the evolution or

105 evolutionary history of facultative mutualisms between fishes and anemones, these species

106 exhibit notable phylogenetic diversity (Randall & Fautin 2002), which suggests that these

107 relationships have evolved on multiple occasions. Several experimental, long-term monitoring

108 and observational studies on a variety of fish-anemone partnerships also provide direct and

109 indirect evidence in support of the hypothesis that protection from predators is a key benefit

110 of, at least, some of these relationships (e.g. Mariscal 1970a; Elliott 1992; Holbrook & Schmitt

111 2002; Buston 2003b). Taken together, these studies indicate that fish-anemone mutualisms may

112 provide a useful model to test whether predation can explain recurrent patterns of mutualism

113 evolution.

114

115 In this study, we first conducted a series of phylogenetic analyses to investigate: i) the

116 evolutionary history of fish-anemone mutualisms among both facultative and obligate fish

117 clades, and ii) whether fish body size or life-history stage could explain patterns of anemone

118 associations. Considering that many predatory fishes are gape-limited (Goatley & Bellwood

119 2016) we expected that these relationships have evolved on multiple occasions and that fish

120 body size, rather than life-history stage should determine patterns of fish-anemone mutualism

121 evolution: smaller-bodied species should associate with anemones throughout their lives (i.e.

122 as juveniles and adults) while larger-bodied species should only associate with anemones when

123 they are small (i.e. as juveniles). Second, we carried out a series of field and laboratory

124 experiments on the threespot dascyllus (*Dascyllus trimaculatus*) – a facultative anemone-

125 mutualist – to investigate: i) if body size could explain patterns of anemone associations

126 between individuals under natural conditions, ii) whether smaller individuals were more

127 vulnerable to predation by three common predators compared to larger counterparts, and iii)

128 whether associations with anemones conferred protective benefits compared to (A) a locally

129 abundant and closely related non-anemone associated comparison species, the surge

130 damselfish (*Chrysiptera brownriggi*), and (B) individuals from the same species that had

131 access to a coral refuge.

132

133 **MATERIALS & METHODS**

134 **Phylogenetic and ancestral-state analyses**

135 To investigate the evolutionary history of anemone-associations in fishes, we first compiled

136 records of fishes that associate with anemones from the literature (table S1). We recorded two

137 pieces of information for each anemone-associated fish species: i) the ontogenetic stage at

138 which the species had been observed to associate with anemones, and ii) whether the

139 association was facultative or obligate. This resulted in a dataset of 88 species across 16

140 families that could be placed into three possible character states: i) "juvenile-only facultative"

141 ($n = 19$) – facultative use of anemones only recorded in juvenile individuals (average max body

142 size $= 332.0$ mm ± 34.8 mm); ii) "facultative" ($n = 22$) – facultative use of anemones recorded

143 in both juvenile and adult individuals (average max body size $= 82.9 \pm 6.63$ mm); iii) "obligate"

144 ($n = 29$) – obligate use of anemones recorded across life history stages (average max body size

145 $= 126.0$ mm ± 4.87 mm). We also included "non-mutualist" ($n = 1823$) as an additional

146 character state, which comprised species that had not previously been recorded to associate

147 with anemones but were sampled in the phylogeny and found within families that had at least

148 one anemone-associated representative (average max body size $= 239$ mm ± 6.63 mm). The

149 ontogenetic stage of anemone-association was unknown for 18 of the 88 species (average max

150 body size = 113.0 mm ± 7.6 mm) (table S1).

151

152 To investigate the number of independent origins of fish-anemone mutualisms, the number of

153 transitions between states and whether there was an effect of size on life-stage of anemone-

154 association when compared to non-anemone associated counterparts, we used a recently

155 published time-calibrated fish 'tree of life' (Rabosky *et al.* 2018), which we pruned down to

156 the most inclusive clade that represented all families that contain species that have been

157 recorded to display mutualistic relationships with anemones. The resulting phylogeny included

158 16 families within the Acanthopterygii. There were 18 species in our dataset that were not

159 represented in the pruned phylogeny. To incorporate uncertainty regarding the placement of

160 these unsampled tips, we generated a set of 1000 phylogenies and grafted the unsampled tips

161 to their most likely sister species or clade based on previously published information (table

162 S2). The tips were grafted with a branching point drawn at random along the grafted lineage

163 using the *bind.tip* function in the R package PHYTOOLS v0.6-2 20 (Revell 2012). The final

164 phylogeny included 1956 species.

165

166 Stochastic character mapping (Huelsenbeck *et al.* 2003) was used to infer possible histories of

167 anemone use across the phylogeny. First, we tested and compared models which allowed

168 reversible or irreversible transitions between mutualist states. In each analysis the transition

169 (Q) matrix was fitted to the tree and data using the 'fitML()' function of PHYTOOLS. For each

170 model, 1000 stochastic character histories were simulated across the 1000 set of grafted trees

171 (one map per tree). The irreversible model performed slightly better when compared by AIC

172 score (IRR: 675.22, SYM:676.56); but AIC weights could not separate the two models. To

173 allow for possible reversal from mutualist to non-mutualist, we chose the SYM model. The

174 1000 simulated character histories were summarised to explore the number of independent

175 origins of anemone use across the tree and number of transition between states. This was done

176 for three variations on the mutualism state coding: one where the unknown life history stage

177 mutualist species were coded as "facultative" as adults, one where there were coded as

178 "unknown", and one where equal probability were given to the species being facultative as

179 juvenile-only or as juveniles and adults. The final coding scheme allowed the stochastic

180 analysis to predict which state the unknown facultative tips were likely to be in.

181

182 Finally, to investigate the effect of body size on association type, we retrieved adult body

183 lengths from fishbase.org. Out of the 1956 species tree (Rabosky et al. 2018) there were 50

184 species for which body size data were not available. As these species were non-mutualists and

185 phylogenetically distant from any transition to any mutualism category, we pruned them from

186 the tree prior to analyses. We used phylogenetic generalised least squares regression (PGLS)

187 to explore whether maximum adult body size (TL) varied among species that display

188 mutualism behaviour with anemones as juveniles or as juveniles and adults, and whether there

189 was a relationship between maximum body size and association type (facultative, juvenile-only

190 facultative, obligate and unknown). As more closely related species are assumed to exhibit

191 more similar traits (Revell 2010), PGLS takes into account the expected covariance structure

192 of residuals for a given phylogeny. The correlation structure was first derived from the

193 phylogenetic tree assuming Brownian motion model of evolution and a maximum likelihood

194 estimate of Pagel's λ (Pagel 1997) using the *corPagel* function of the R package *ape* (Paradis

195 *et al.* 2004). The PGLS was then run using the *gls* function in the R package *nlme* (Pinheiro *et*

196 *al.* 2013).

197

198 **Field and aquaria studies**

Study Site and Species

200 Field and aquaria studies were conducted at Moorea (17°29'S, 149°49'W), French Polynesia,

201 between January and February 2016. Transects and animal collection were conducted on the

202 shallow reef flats (depth: 2 – 12 m) surrounding the island; aquaria experiments were conducted

203 at CRIOBE Research Station. Threespot dascyllus, surge damselfish, arc-eye hawkfish

204 (*Paracirrhites arcatus*), and speckled sandperch (*Parapercis hexophtalma*) were collected on

205 snorkel and SCUBA using anaesthetic clove oil solution (15% clove oil; 15% ethanol; 70%

206 seawater), hand nets and small barrier nets. Anemones (*Heteractis magnifica*) and corals

207 (*Pocillopora eydouxi*) were collected from the same shallow reefs. Smallmouth squirrelfish

208 (*Sargocentron microstoma*) were opportunistically collected as by-catch from a crest net.

209

210 Specimen collection was conducted on a rolling schedule to meet experimental demands.

211 Dascyllus and surge damselfish were sorted into 4 size categories for use in field and laboratory

212 studies: "recruit" (0 – 2 cm), "juvenile" (2.1 – 4 cm), "small adult" (4.1 – 6 cm), and "large

213 adult" (> 6.1 cm). Following collection and prior to use in aquaria experiments; dascyllus,

214 surge damselfish, anemones ($n = 9$, all ~ 30 cm diameter) and corals ($n = 12$, all ~ 30 cm

215 diameter) were held together in a 630 L tank (350 * 60 * 30 cm). Anemones were fed a 1 cm^2

216 piece of prawn every 48 h. Dascyllus and surge damselfishes were fed *ad libitum* with

217 granulated fish food (Tetra) and newly hatched *Artemia nauplii*. Anemones and corals were

218 placed in experimental tanks a minimum of 48 h prior to use in experiments and were switched

219 indiscriminately between tanks prior to each trial to control for any potential specimen effects.

220 Potential prey (dascyllus and surge damselfishes) were placed in tanks 30 minutes prior to the

221 start of a trial to allow time for acclimation prior to the addition to a predator (hawkfish,

222 sandperch, or squirrelfish) and the trial beginning. Each fish was only used in one experimental

223 trial before being released back onto the reef from which it was collected (predation trial

224 methods similar to Cortesi *et al.* 2015). Hawkfishes, sandperches, and squirrelfishes were held

225 together in two 843 L holding tanks (150 * 75 * 75) and were fed *ad libitum* with small pieces

226 of prawn. Twenty-four hours prior to use in experiments they were transferred to individual 45

227 L holding tanks (50 * 30 * 30 cm) where they were not fed to help control for satiation level.

228 Tanks were cleaned between each trial.

229

230 ***Transects***

231 To investigate patterns of anemone-associations by fishes, patterns of habitat use by dascyllus

232 and surge damselfish, and whether anemone-associations varied through ontogeny in

233 dascyllus, we conducted thirty 25 m transects along the shallow reef flats at Tema'e Bay

234 (17°29'41.4" S, 149°45'07.6" W). The observer noted the habitat over which all dascyllus

235 (recruits, juveniles, small adults and large adults), surge damselfish (recruits, juvenile, small

236 adults, large adults) and orange-fin anemonefish (*Amphiprion chrysopterus*) were at the time

237 of first observation as well as the total number of anemones along each transect. Fishes and

238 anemones were recorded if they were sighted within 1.5 m either side of the transect tape, and

239 each transect was run at approximately 3 m intervals perpendicular to the coastline. Of the 133

240 anemones sighted during the transects, 124 (93.23 %) hosted threespot dascyllus, 9 (6.77 %)

241 hosted orange-fin anemonefish and 9 (6.77 %) hosted no fish. Orange-fin anemonefishes were

242 always found on anemones that also hosted threespot dascyllus, and surge damselfish were

243 never found in association with anemones. Hawkfish, sandperch and squirrelfish were all

244 commonly observed during transects, but their abundances were not recorded. We used a

245 generalized linear mixed model (GLMM) with a Poisson distribution to investigate how

246 anemone-associations varied through ontogeny in dascyllus in the R package *lme4* (Bates &

247 Maechler 2009). The full model included substrate type and fish size as fixed effects, and

248 transect ID as a random effect. We used a zero-inflated generalized linear mixed model with a

249 Poisson distribution to investigate whether fishes of different sizes associated more with

250 anemones of different sizes in the R package *glmmTMB* (Magnusson *et al.* 2018). The full

251 model included anemone size and fish size class as fixed effects, and transect ID as a random

252 effect.

253

254 ***Predation Experiment #1***

255 To investigate whether predator pressure varied according to prey body size in dascyllus, we

256 conducted a prey-choice experiment using three common predators of juvenile fishes.

257 Hawkfish (SL = 7.84 ± 0.47 cm, range = 6.1 – 8.7 cm), sandperch (SL = 12.4 ± 1.05 cm, range

258 = 9.3 – 15.8 cm) and squirrelfish (SL = 16.32 ± 0.15 cm, range = 15.8 – 16.6 cm) were used as

259 the predators. We conducted preliminary trials to confirm that the smallest predator individuals

260 from each species were able to consume the largest prey individuals from the recruit and

261 juvenile size classes. We also included small adults in our trials to investigate whether these

262 predators were a threat to larger dascyllus individuals.

263

264 Experimental tanks ($n = 6$) contained equal amounts of rubble substrate. One hour prior to each

265 experimental trial, a dascyllus from each size class (recruit, juvenile and small adult) was

266 placed into an experimental tank. Prey size within size class did not differ across predator

267 treatments ($\chi_4^2 = 467.05, P = 0.88$). The trial began when one of the predators was added to

268 a tank ($n = 10$ trials for each of the three predator species). Each trial was checked after 3 h and

269 then approximately every hour until either a fish was captured or 24 h passed. If the latter

270 occurred, all fish were replaced and the trial was re-run (this occurred in two of 32 trials).

271 Following each trial, the remaining fish were removed from the experimental tank and placed

272 in a holding tank prior to release. We used a generalized linear mixed model with a binomial

273 distribution to investigate whether predators preferentially targeted small, medium or large

274   prey items. The full model included prey size, predator species and their interaction as fixed

275   effects, and trial as a random effect. Main effects were tested only if the relevant interaction

276   was not significant. We used a generalized linear mixed model with a Poisson distribution to

277   investigate whether prey size varied within size classes across predator treatments. The full

278   model included prey size category and predator species as fixed effects and trial as a random

279   effect.

280

281   ***Predation experiment #2***

282   To investigate whether predators would preferentially target prey not associated with an

283   anemone over those with an anemone, we conducted a predation experiment using surge

284   damselfishes (non-anemone-associated) and dascyllus (anemone-associated). Each

285   experimental tank ($n = 8$) contained one coral, one anemone and equal amounts of rubble

286   substrate. One hour prior to each experimental trial ($n = 20$), we placed 10 dascyllus and 10

287   surge damselfish recruits in the tank to acclimate. Following acclimation, a hawkfish (SL =

288   7.75 ± 0.27 cm, range = $6.1 - 8.8$ cm) was added and the experiment was left to run for 24 h.

289   After the 24 h experimental period, the hawkfish was removed from the tank and the remaining

290   damselfish and dascyllus were counted, removed from the tank and placed into the holding

291   tank prior to being released. We used a generalised linear model with a gamma distribution to

292   investigate survival rates of surge damselfish and dascyllus individuals. The full model

293   included prey species as a fixed effect.

294

295   ***Predation experiment #3***

296   Finally, we investigated whether the presence of anemones decreased the likelihood of juvenile

297   dascyllus being captured. Each experimental tank ($n = 8$) contained an equal amount of rubble

298   substrate as well as either an anemone or a coral. Each experimental trial comprised an

299   anemone and coral treatment ($n = 20$ trials). Prior to each experimental treatment, ten dascyllus

300   recruits were placed into the tank and allowed 1 h acclimation, after which a hawkfish (SL =

301   7.79 ± 0.23 cm, range = $6.2 – 8.8$ cm) was added to the tank and the experiment was left to run

302   for 24 h. After the 24 h experimental period, the hawkfish was removed from the tank and the

303   remaining dascyllus were counted, removed from the tank and placed into the holding tank

304   prior to being released. We used a generalized linear model with a gamma distribution to

305   investigate survival rates of dascyllus individuals. The full model included refuge type as a

306   fixed effect.

307

308   **RESULTS**

309   **Evolutionary History of Fish-Anemone Mutualisms**

310   We found that fish-anemone mutualisms independently evolved on at least 55 occasions across

311   16 families over the past 60 million years (MY) (Fig. 1). Our results indicate that obligate

312   anemone associations evolved on one occasion approximately 22 MYA, while facultative

313   associations evolved on 54 occasions over the past 60 MY and show no congruent pattern in

314   their temporal emergence (Fig. 2A-C). Stochastic character mapping performed on a modified

315   time-calibrated fish phylogeny (Rabosky *et al.* 2018) indicates that anemone-associations

316   evolved directly from non-anemone-associated ancestors in both facultative and obligate clades

317   and that this behaviour has been lost on at least 2 occasions among facultative lineages (Fig.

318   2D). The same number of transitions was recorded when species with an unknown life-history

319   stage of anemone association (18 species) were coded as "unknown" rather than "facultative".

320   Further, when we allowed the stochastic mapping analysis to predict whether unknown

321   facultative taxa were mutualists as juveniles only or as juveniles and adults (i.e. facultative), it

322   definitively predicted a tip to be facultative in only one instance (Moluccan cardinal fish

323   *Ostorhinchus moluccensis* – 97% of stochastic maps). For all other unknown taxa, the

324   prediction was equivocal ($<60\%$ of maps in either stage) due to the lack of closely related taxa

325   that are also associated with anemones. Given the lack of predictability and the distribution of

326   body sizes in the unknown facultative taxa, we proceeded with analyses where unknowns were

327   coded as "facultative".

328

329   Using published records of the life stage(s) at which fishes associate with anemones (table S1),

330   we tested whether the adult body size of fishes that associate with anemones correlates with

331   the life history stage at which they maintain a mutualism. There were significant differences in

332   maximum body size among mutualist categories ($F_{3,1902} = 3.62$, $P = 0.012$). We found that,

333   within families that included both anemone-associated and non-anemone-associated species,

334   lineages that have evolved a facultative association as juveniles-only (332 ± 34.8 mm) were

335   significantly larger than non-mutualist lineages (239 ± 6.63 mm) (coefficient: 0.06, $t_{1902} = 2.9$,

336     $P = 0.003$), while lineages that display facultative associations as both juveniles and adults

337   (96.6 ± 6.86 mm) were not significantly smaller than non-mutualist lineages (coefficient: -

338   0.02, $t_{1902} = -1.35$, $P = 0.17$), and obligately associated lineages (126 ± 4.87 mm) were also not

339   significantly different in size from non-mutualists (coefficient: 0.05, $t_{1902} = 0.75$, $P = 0.45$)

340   (Fig. 2E). These results did not change when we excluded the obligate anemonesfishes ($F_{2, 1874}$

341     $= 6.17$, $P = 0.002$) from our analyses, when species with an unknown life-history stage of

342   anemone association were excluded from our analysis ($F_{3, 1884} = 4.17$, $P = 0.006$), or when we

343   removed the 18 mutualist species that were grafted to the molecular phylogeny ($F_{3, 1884} = 6.08$,

344     $P = 0.0004$).

345

346   **Field and Laboratory Studies: Does Predation Select Anemone Mutualisms?**

347   Our transect data revealed that there was a significant interaction between substrate type and

348   fish size ($\chi_3^2 = 452.57, P < 0.0001$, Fig. 3): whilst recruit (< 2 cm TL, $n = 326$) and juvenile

349   ($2 - 4$ cm TL, $n = 387$) dascyllus, which were both found almost exclusively in association

350   with anemones, did not differ in their substrate use (coefficient: 1.35, $z = 1.24$, $P = 0.22$), there

351   was a progressive decrease in the use of the anemone in the larger dascyllus size classes (small

352   adults, $4 - 6$ cm TL, $n = 240$; coefficient: 3.39, $z = 3.32$, $P = 0.0009$; and large adults, > 6 cm

353   TL, $n = 169$, coefficient: 6.60, $z = 1.01$, $P < 0.0001$). Our analysis also revealed an interaction

354   between the abundance of fishes within a particular size class and anemone size ($\chi_3^2 = $

355   $27.96, P < 0.0001$). However, with the exception of juvenile fishes, which were found in

356   significantly higher abundances on larger anemones (coefficient: 0.23, $z = 4.24$, $P < 0.0001$),

357   there were no significant interactions between fish size and anemone size in small adults

358   (coefficient: 0.09, $z = 1.37$, $P = 0.72$) or large adults (coefficient: 0.04, $z = 0.21$, $P = 0.84$),

359   indicating no evidence of larger fishes associating more frequently with larger anemones.

360

361   When investigating whether three different predators (arc-eye hawkfish, $n = 10$; speckled

362   sandperch, $n = 10$; and smallmouth squirrelfish, $n = 10$) preferentially targeted small, medium

363   or large dascyllus, we found no difference in prey body size preference ($\chi_2^2 = 57.46$, $P = 0.89$).

364   Across these three predators, recruit fishes were removed significantly more than both juvenile

365   and small adult dascyllus by all three predator species ($\chi_1^2 = 57.46$, $P < 0.001$) (Fig. 4a, b, c).

366   When ten dascyllus and ten surge damselfish recruits were placed in a tank with an anemone,

367   a coral, coral rubble, and a hawkfish predator ($n = 20$ trials), significantly more dascyllus

368   survived compared to surge damselfishes ($F_{1, 39} = 28.9$, $P < 0.0001$, Fig. 4d). When ten

369   dascyllus recruits were placed in a tank with coral rubble, a hawkfish predator and either a

370   coral ($n = 20$ trials) or an anemone ($n = 20$ trials), significantly more dascyllus survived when

371   an anemone refuge was available ($F_{1, 39} = 31.98$, $P < 0.0001$, Fig. 4e). We also detected no

372 significant differences when we compared survival of dascyllus with access to a coral refuge

373 to that of surge damselfishes ($W = 161$, $P = 0.276$), nor in total predation across these trials (W

374 $= 513.5$, $P = 0.063$).

375

376 **DISCUSSION**

377 Predation has been shown to select protective mutualisms in a variety of species-specific case-

378 studies (Bronstein 1994; Hay *et al.* 2004; Bascompte & Jordano 2007), as well as regulate

379 mutualism dynamics between interacting species (Palmer *et al.* 2008; Canestrari *et al.* 2014).

380 However despite being an intuitive expectation, no prior study appears to have directly

381 investigated whether predation might also select globally generalizable patterns of convergence

382 in interspecies mutualisms (Doebeli & Knowlton 1998; Toby Kiers *et al.* 2010; Bittleston *et*

383 *al.* 2016). When considering fishes that form both facultative and obligate mutualisms with

384 anemones, our phylogenetic and transition-state analyses indicate that: i) these relationships

385 have evolved on numerous occasions, ii) while obligate associations with anemones evolved

386 on one occasion, facultative associations have continuously emerged and disappeared over the

387 past 60 MY, and iii) maximum adult body size is associated with the life-history stage at which

388 fishes have been recorded to associate with anemones, with smaller-bodied species, or

389 individuals within species, associating with anemones more than their larger counterparts. To

390 investigate the causality of these patterns, we conducted field transects and a series of predation

391 experiments on the threespot dascyllus as a case study, which show that: i) there is an inverse

392 relationship between fish body size and its likelihood of being observed in association with an

393 anemone, and no evidence that larger fishes seek out larger anemones, ii) three common

394 piscivorous predators all preferentially target smaller prey over larger prey, iii) predators

395 preferentially target prey from species that do not associate with anemones over those that do,

396 and iv) within a species, individuals that associate with anemones are less likely to be preyed

397 on compared to those that only have access to a coral refuge. Considering that several other

398 species-specific case studies have suggested that protection from predators is a benefit of these

399 relationships in both facultative (Elliott 1992) and obligate (Mariscal 1970b) fish lineages, our

400 results strongly suggest that selection by predators has been instrumental in the recurrent

401 convergent evolution of fish-anemone mutualisms, and that this pattern of evolutionary

402 convergence is globally generalizable.

403

404 Our results highlight that fish-anemone mutualisms are geographically and phylogenetically

405 common relationships. When considering facultative and obligate mutualisms together, our

406 results show that these relationships have evolved on at least 55 occasions across 16 families

407 (Fig. 1), indicating that over a quarter (27%) of coral reef associated fish families contain at

408 least one representative that has been recorded to form mutualisms with anemones. While it is

409 difficult to compare facultative versus obligate mutualisms, given that obligate relationships

410 are only represented in the monophyletic anemonefishes, there does appear to be some notable

411 differences between the two. Facultative mutualisms appear to be highly labile, behaviourally

412 opportunistic relationships that have evolved on numerous occasions in geographically

413 disparate locations, such as the Mediterranean, West-Atlantic, and Indo-Pacific. Once they

414 emerge, they show little evidence of subsequent diversification, with exception of some small

415 clades within the Pomacentridae, Apogonidae and Labrisomidae (Fig. 1). Obligate mutualisms,

416 in contrast, are associated with accompanying specialized phenotypes, which may contribute

417 to them being rarer and less labile compared to facultative mutualisms (Santini & Polacco

418 2006). The emergence of obligate mutualisms with anemones also appears to have preceded a

419 period of rapid diversification (Cowman & Bellwood 2011; Litsios *et al.* 2012). One question

420 concerns why obligate mutualisms have evolved so rarely compared to their facultative

421 counterparts. However, this result is perhaps unsurprising given that the creation of reef

422 habitats throughout the Indo-Australian Archipelago during the Oligocene and Miocene

423 underpinned high rates of cladogenesis and niche-specialization (Cowman & Bellwood 2011).

424 For example, cleaning and corallivory are relatively common and geographically diverse life-

425 history strategies in coral reef fishes, but similar to anemone mutualisms, obligate

426 representatives are also primarily restricted to the Indo-Pacific and evolved around this period

427 (Cowman & Bellwood 2011; Frédérich *et al.* 2013; Baliga & Law 2016). Overall, despite

428 facultative and obligate mutualisms appearing to exhibit at least some differences, the general

429 lack of a pattern in the temporal emergence of these relationships over the past 60 MY as well

430 as their geographic and phylogenetic diversity suggests that a pervasive ecological pressure,

431 rather than a discrete biological or geological event, may best explain the repeated evolution

432 of this trait.

433

434 The high fitness costs that are experienced by smaller fishes, regardless of their phylogenetic

435 position or life-history stage, makes fish-anemone mutualisms well-suited to explore the

436 ecological pressures that can select the recurrent evolution of interspecies mutualisms. The life-

437 history of many reef-associated fishes comprises a dispersive larval phase followed by more

438 sedentary juvenile and adult phases. Larval fishes face a severe predation bottleneck as they

439 return to the reef (Almany & Webster 2006), with a recent meta-analysis estimating an average

440 daily mortality rate of approximately 30% of predator-naïve recruits per day across species

441 during this time (Goatley & Bellwood 2016). While the likelihood of predation dramatically

442 decreases as experience with predators and/or body-size increases (Munday & Jones 1998;

443 Depczynski & Bellwood 2006; Mitchell *et al.* 2013; Goatley & Bellwood 2016), mortality risk

444 still averages approximately 3-8% per day until total length reaches approximately 43 mm,

445 after which it stabilizes around 0.2% per day (Goatley & Bellwood 2016). When comparing

446 across species, our phylogenetic analysis shows that maximum adult body size varies

447   significantly with the stage at which species maintain mutualisms with anemones: species that

448   associate with anemones as both juveniles and adults tend to be smaller bodied species, and

449   species that associate with anemones exclusively as juveniles are significantly larger than non-

450   mutualists as adults (Fig. 2E). Our field transects and aquaria experiments corroborate these

451   patterns, showing that smaller fishes associate more with anemones compared to larger fishes,

452   and that they are the primary beneficiaries of these mutualisms. It is interesting that our transect

453   data, as well as data from other mutualism systems, indicates that these relationships weaken

454   once individuals reach approximately 40 mm in length. For example, several species of tubelip

455   wrasse (*Diproctacanthurus xanthurus* and *Labropsis alleni*) have been observed to exhibit

456   cleaning behaviours, which are associated with decreased predation risk (Côté 2000) until they

457   reach approximately 35-50 mm in length (Cole 2010; Grutter & Feeney 2016) and similar to

458   fish-anemone associations, numerous small fishes obtain protective benefits through

459   associations with other sessile organisms, such as corals or urchins, to whom they provide

460   nutrition through their excrement (Liberman *et al.* 1995; Vagelli & Erdmann 2002). Therefore,

461   while our study focused on fish-anemone mutualisms, our results may indicate that the gape-

462   limitations inherent to many predatory fishes might help explain and unify diverse studies

463   regarding the evolution of size-dependent protective mutualisms, especially in marine

464   environments.

465

466   Our key insight is that, in addition to affecting mutualism evolution in species-specific case

467   studies and regulating mutualism dynamics between interacting species (Palmer *et al.* 2008;

468   Canestrari *et al.* 2014), the pressure imposed on prey by predators might also explain global

469   patterns of convergence of interspecies mutualism evolution. While protective mutualisms are

470   near ubiquitous components of almost all ecosystems (Bronstein 1994; Hay *et al.* 2004;

471   Bascompte & Jordano 2007), the size-dependent nature of these relationships in marine

472 environments appears to make them well-suited for discerning the pressures selecting

473 protective mutualisms across a wide range of species compared to their terrestrial counterparts,

474 where predation pressure is less linked to prey body size (Chamberlain & Holland 2009; Trager

475 *et al.* 2010). We emphasise the importance of understanding how external pressures can

476 influence convergent patterns of interspecies interactions and the fundamental role that

477 predation has played in shaping the evolution of the world's biodiversity.

478

486

487 **Author Contributions** W.E.F and R.M.B conceived the study, with important contributions

488 from A.M and P.F.C; phylogenetic comparative analyses and ancestral state reconstructions

489 were conducted by P.F.C, with assistance from W.E.F, R.M.B, J.D.J.G and A.M; field and

490 laboratory studies of fish behaviour were conducted by W.E.F and R.M.B, with assistance from

491 L.N.J, M.B, and D.L; statistical analyses were conducted by W.E.F, A.M and P.F.C; W.E.F

492 wrote the manuscript with input from all authors.

493

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633

Figure Legends

Fig. 1. Representative stochastic character map of anemone use on a pruned, time calibrated phylogeny (Rabosky *et al.* 2018) for 1956 fish species. Concentric circles indicate time (MY) and the alternating colours (grey and black) in the peripheral ring indicates fish Families/Orders where mutualistic behaviour with anemones has been recorded by member species.

Fig. 2. Percent of state changes recorded from 1000 stochastic character mapping through time (A, B, C, D) and the distribution of species' maximum body sizes across association type and life history stage of recorded anemone association ('facultative' indicates species that have been recorded to partner with anemones as juveniles and adults) (E). Boxplots denote median, quartiles and range; brackets denote sample sizes; asterisks denote significant differences between non-mutualist and juvenile-only facultative, and juvenile-only facultative and facultative.

Fig. 3. Percent of dascyllus individuals observed in association with anemones across four size classes on the reef flats at Tema'e Bay, Moorea.

Fig. 4. Likelihood of dascyllus individuals from different size classes surviving (percent ± standard error) when exposed to three common piscivores (A, B, C). Juvenile dascyllus and surge damselfish survival (percent ± standard error) when exposed to a hawkfish predator for 24 h with both an anemone and coral refuge (D). Juvenile dascyllus survival (percent ± standard error) when exposed to a hawkfish predator for 24 h with either an anemone or coral refuge (E).