Title: Predation drives recurrent convergence of an interspecies mutualism

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**Predation drives recurrent convergence of an interspecies mutualism**

William E. Feeney1,2,†,\*, Rohan M. Brooker2,3,†, Lane N. Johnston2, James D. J. Gilbert4, Marc Besson5,6, David Lecchini5, Danielle L. Dixson2, Peter F. Cowman7, Andrea Manica8

**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 fishes gain benefits such as increased access to nutrition or increased aeration (Szczebak *et al.* 2013; Lim *et al.* 2016), protection from predators is widely credited as a key benefit that anemonefishes receive through their associations with anemones (reviewed in Fautin 1991). However, given the obligate nature of these relationships, this has proven difficult to conclusively demonstrate. In contrast, while no prior research has investigated the evolution or evolutionary history of facultative mutualisms between fishes and anemones, these species exhibit notable phylogenetic diversity (Randall & Fautin 2002), which suggests that these relationships have evolved on multiple occasions. Several experimental, long-term monitoring and observational studies on a variety of fish-anemone partnerships also provide direct and indirect evidence in support of the hypothesis that protection from predators is a key benefit of, at least, some of these relationships (e.g. Mariscal 1970a; Elliott 1992; Holbrook & Schmitt 2002; Buston 2003b). Taken together, these studies indicate that fish-anemone mutualisms may provide a useful model to test whether predation can explain recurrent patterns of mutualism evolution.

In this study, we first conducted a series of phylogenetic analyses to investigate: i) the evolutionary history of fish-anemone mutualisms among both facultative and obligate fish clades, and ii) whether fish body size or life-history stage could explain patterns of anemone associations. Considering that many predatory fishes are gape-limited (Goatley & Bellwood 2016) we expected that these relationships have evolved on multiple occasions and that fish body size, rather than life-history stage should determine patterns of fish-anemone mutualism evolution: smaller-bodied species should associate with anemones throughout their lives (i.e. as juveniles and adults) while larger-bodied species should only associate with anemones when they are small (i.e. as juveniles). Second, we carried out a series of field and laboratory experiments on the threespot dascyllus (*Dascyllus trimaculatus*) – a facultative anemone-mutualist – to investigate: i) if body size could explain patterns of anemone associations between individuals under natural conditions, ii) whether smaller individuals were more vulnerable to predation by three common predators compared to larger counterparts, and iii) whether associations with anemones conferred protective benefits compared to (A) a locally abundant and closely related non-anemone associated comparison species, the surge damselfish (*Chrysiptera brownriggi*), and (B) individuals from the same species that had access to a coral refuge.

**MATERIALS & METHODS**

**Phylogenetic and ancestral-state analyses**

To investigate the evolutionary history of anemone-associations in fishes, we first compiled records of fishes that associate with anemones from the literature (table S1). We recorded two pieces of information for each anemone-associated fish species: i) the ontogenetic stage at which the species had been observed to associate with anemones, and ii) whether the association was facultative or obligate. This resulted in a dataset of 88 species across 16 families that could be placed into three possible character states: i) “juvenile-only facultative” (*n* = 19) – facultative use of anemones only recorded in juvenile individuals (average max body size = 332.0 mm ± 34.8 mm); ii) “facultative” (*n* = 22) – facultative use of anemones recorded in both juvenile and adult individuals (average max body size = 82.9 ± 6.63 mm); iii) “obligate” (*n* = 29) – obligate use of anemones recorded across life history stages (average max body size = 126.0 mm ± 4.87 mm). We also included “non-mutualist” (*n =* 1823) as an additional character state, which comprised species that had not previously been recorded to associate with anemones but were sampled in the phylogeny and found within families that had at least one anemone-associated representative (average max body size = 239 mm ± 6.63 mm). The ontogenetic stage of anemone-association was unknown for 18 of the 88 species (average max body size = 113.0 mm ± 7.6 mm) (table S1).

To investigate the number of independent origins of fish-anemone mutualisms, the number of transitions between states and whether there was an effect of size on life-stage of anemone-association when compared to non-anemone associated counterparts, we used a recently published time-calibrated fish ‘tree of life’ (Rabosky *et al.* 2018), which we pruned down to the most inclusive clade that represented all families that contain species that have been recorded to display mutualistic relationships with anemones. The resulting phylogeny included 16 families within the Acanthopterygii. There were 18 species in our dataset that were not represented in the pruned phylogeny. To incorporate uncertainty regarding the placement of these unsampled tips, we generated a set of 1000 phylogenies and grafted the unsampled tips to their most likely sister species or clade based on previously published information (table S2). The tips were grafted with a branching point drawn at random along the grafted lineage using the *bind.tip* function in the R package PHYTOOLS v0.6-2 20(Revell 2012). The final phylogeny included 1956 species.

Stochastic character mapping(Huelsenbeck *et al.* 2003) was used to infer possible histories of anemone use across the phylogeny. First, we tested and compared models which allowed reversible or irreversible transitions between mutualist states. In each analysis the transition (Q) matrix was fitted to the tree and data using the ‘fitML()’ function of PHYTOOLS. For each model, 1000 stochastic character histories were simulated across the 1000 set of grafted trees (one map per tree). The irreversible model performed slightly better when compared by AIC score (IRR: 675.22, SYM:676.56); but AIC weights could not separate the two models. To allow for possible reversal from mutualist to non-mutualist, we chose the SYM model. The 1000 simulated character histories were summarised to explore the number of independent origins of anemone use across the tree and number of transition between states. This was done for three variations on the mutualism state coding: one where the unknown life history stage mutualist species were coded as “facultative” as adults, one where there were coded as “unknown”, and one where equal probability were given to the species being facultative as juvenile-only or as juveniles and adults. The final coding scheme allowed the stochastic analysis to predict which state the unknown facultative tips were likely to be in.

Finally, to investigate the effect of body size on association type, we retrieved adult body lengths from fishbase.org. Out of the 1956 species tree (Rabosky et al. 2018) there were 50 species for which body size data were not available. As these species were non-mutualists and phylogenetically distant from any transition to any mutualism category, we pruned them from the tree prior to analyses. We used phylogenetic generalised least squares regression (PGLS) to explore whether maximum adult body size (TL) varied among species that display mutualism behaviour with anemones as juveniles or as juveniles and adults, and whether there was a relationship between maximum body size and association type (facultative, juvenile-only facultative, obligate and unknown). As more closely related species are assumed to exhibit more similar traits(Revell 2010), PGLS takes into account the expected covariance structure of residuals for a given phylogeny. The correlation structure was first derived from the phylogenetic tree assuming Brownian motion model of evolution and a maximum likelihood estimate of Pagel’s (Pagel 1997)using the *corPagel* function of the R package *ape* (Paradis *et al.* 2004). The PGLS was then run using the *gls* function in the R package *nlme* (Pinheiro *et al.* 2013).

**Field and aquaria studies**

***Study Site and Species***

Field and aquaria studies were conducted at Moorea (17°29’S, 149°49’W), French Polynesia, between January and February 2016. Transects and animal collection were conducted on the shallow reef flats (depth: 2 – 12 m) surrounding the island; aquaria experiments were conducted at CRIOBE Research Station. Threespot dascyllus,surge damselfish, arc-eye hawkfish (*Paracirrhites arcatus*), and speckled sandperch (*Parapercis hexophtalma*) were collected on snorkel and SCUBA using anaesthetic clove oil solution (15% clove oil; 15% ethanol; 70% seawater), hand nets and small barrier nets. Anemones (*Heteractis magnifica*) and corals (*Pocillopora eydouxi*) were collected from the same shallow reefs. Smallmouth squirrelfish (*Sargocentron microstoma*) were opportunistically collected as by-catch from a crest net.

Specimen collection was conducted on a rolling schedule to meet experimental demands. Dascyllus and surge damselfish were sorted into 4 size categories for use in field and laboratory studies: “recruit” (0 – 2 cm), “juvenile” (2.1 – 4 cm), “small adult” (4.1 – 6 cm), and “large adult” (> 6.1 cm). Following collection and prior to use in aquaria experiments; dascyllus, surge damselfish, anemones (*n* = 9, all ~ 30 cm diameter) and corals (*n* = 12, all ~ 30 cm diameter) were held together in a 630 L tank (350 \* 60 \* 30 cm). Anemones were fed a 1 cm2 piece of prawn every 48 h. Dascyllus and surge damselfishes were fed *ad libitum* with granulated fish food (Tetra) and newly hatched *Artemia nauplii*. Anemones and corals were placed in experimental tanks a minimum of 48 h prior to use in experiments and were switched indiscriminately between tanks prior to each trial to control for any potential specimen effects. Potential prey (dascyllus and surge damselfishes) were placed in tanks 30 minutes prior to the start of a trial to allow time for acclimation prior to the addition to a predator (hawkfish, sandperch, or squirrelfish) and the trial beginning. Each fish was only used in one experimental trial before being released back onto the reef from which it was collected (predation trial methods similar to Cortesi *et al.* 2015). Hawkfishes, sandperches, and squirrelfishes were held together in two 843 L holding tanks (150 \* 75 \* 75) and were fed *ad libitum* with small pieces of prawn. Twenty-four hours prior to use in experiments they were transferred to individual 45 L holding tanks (50 \* 30 \* 30 cm) where they were not fed to help control for satiation level. Tanks were cleaned between each trial.

***Transects***

To investigate patterns of anemone-associations by fishes, patterns of habitat use by dascyllus and surge damselfish, and whether anemone-associations varied through ontogeny in dascyllus, we conducted thirty 25 m transects along the shallow reef flats at Tema’e Bay (17°29’41.4” S, 149°45’07.6” W). The observer noted the habitat over which all dascyllus (recruits, juveniles, small adults and large adults), surge damselfish (recruits, juvenile, small adults, large adults) and orange-fin anemonefish (*Amphiprion chrysopterus*) were at the time of first observation as well as the total number of anemones along each transect. Fishes and anemones were recorded if they were sighted within 1.5 m either side of the transect tape, and each transect was run at approximately 3 m intervals perpendicular to the coastline. Of the 133 anemones sighted during the transects, 124 (93.23 %) hosted threespot dascyllus, 9 (6.77 %) hosted orange-fin anemonefish and 9 (6.77 %) hosted no fish. Orange-fin anemonefishes were always found on anemones that also hosted threespot dascyllus, and surge damselfish were never found in association with anemones. Hawkfish, sandperch and squirrelfish were all commonly observed during transects, but their abundances were not recorded. We used a generalized linear mixed model (GLMM) with a Poisson distribution to investigate how anemone-associations varied through ontogeny in dascyllus in the R package *lme4* (Bates & Maechler 2009). The full model included substrate type and fish size as fixed effects, and transect ID as a random effect. We used a zero-inflated generalized linear mixed model with a Poisson distribution to investigate whether fishes of different sizes associated more with anemones of different sizes in the R package *glmmTMB* (Magnusson *et al.* 2018). The full model included anemone size and fish size class as fixed effects, and transect ID as a random effect.

***Predation Experiment #1***

To investigate whether predator pressure varied according to prey body size in dascyllus, we conducted a prey-choice experiment using three common predators of juvenile fishes. Hawkfish (SL = 7.84 ± 0.47 cm, range = 6.1 – 8.7 cm), sandperch (SL = 12.4 ± 1.05 cm, range = 9.3 – 15.8 cm) and squirrelfish (SL = 16.32 ± 0.15 cm, range = 15.8 – 16.6 cm) were used as the predators. We conducted preliminary trials to confirm that the smallest predator individuals from each species were able to consume the largest prey individuals from the recruit and juvenile size classes. We also included small adults in our trials to investigate whether these predators were a threat to larger dascyllus individuals.

Experimental tanks (*n* = 6) contained equal amounts of rubble substrate. One hour prior to each experimental trial, a dascyllus from each size class (recruit, juvenile and small adult) was placed into an experimental tank. Prey size within size class did not differ across predator treatments (). The trial began when one of the predators was added to a tank (*n* = 10 trials for each of the three predator species). Each trial was checked after 3 h and then approximately every hour until either a fish was captured or 24 h passed. If the latter occurred, all fish were replaced and the trial was re-run (this occurred in two of 32 trials). Following each trial, the remaining fish were removed from the experimental tank and placed in a holding tank prior to release. We used a generalized linear mixed model with a binomial distribution to investigate whether predators preferentially targeted small, medium or large prey items. The full model included prey size, predator species and their interaction as fixed effects, and trial as a random effect. Main effects were tested only if the relevant interaction was not significant. We used a generalized linear mixed model with a Poisson distribution to investigate whether prey size varied within size classes across predator treatments. The full model included prey size category and predator species as fixed effects and trial as a random effect.

***Predation experiment #2***

To investigate whether predators would preferentially target prey not associated with an anemone over those with an anemone, we conducted a predation experiment using surge damselfishes (non-anemone-associated) and dascyllus (anemone-associated). Each experimental tank (*n* = 8) contained one coral, one anemone and equal amounts of rubble substrate. One hour prior to each experimental trial (*n* = 20), we placed 10 dascyllus and 10 surge damselfish recruits in the tank to acclimate. Following acclimation, a hawkfish (SL = 7.75 ± 0.27 cm, range = 6.1 – 8.8 cm) was added and the experiment was left to run for 24 h. After the 24 h experimental period, the hawkfish was removed from the tank and the remaining damselfish and dascyllus were counted, removed from the tank and placed into the holding tank prior to being released. We used a generalised linear model with a gamma distribution to investigate survival rates of surge damselfish and dascyllus individuals. The full model included prey species as a fixed effect.

***Predation experiment #3***

Finally, we investigated whether the presence of anemones decreased the likelihood of juvenile dascyllus being captured. Each experimental tank (*n* = 8) contained an equal amount of rubble substrate as well as either an anemone or a coral. Each experimental trial comprised an anemone and coral treatment (*n* = 20 trials). Prior to each experimental treatment, ten dascyllus recruits were placed into the tank and allowed 1 h acclimation, after which a hawkfish (SL = 7.79 ± 0.23 cm, range = 6.2 – 8.8 cm) was added to the tank and the experiment was left to run for 24 h. After the 24 h experimental period, the hawkfish was removed from the tank and the remaining dascyllus were counted, removed from the tank and placed into the holding tank prior to being released. We used a generalized linear model with a gamma distribution to investigate survival rates of dascyllus individuals. The full model included refuge type as a fixed effect.

**RESULTS**

**Evolutionary History of Fish-Anemone Mutualisms**

We found that fish-anemone mutualisms independently evolved on at least 55 occasions across 16 families over the past 60 million years (MY) (Fig. 1). Our results indicate that obligate anemone associations evolved on one occasion approximately 22 MYA, while facultative associations evolved on 54 occasions over the past 60 MY and show no congruent pattern in their temporal emergence (Fig. 2A-C). Stochastic character mapping performed on a modified time-calibrated fish phylogeny (Rabosky *et al.* 2018) indicates that anemone-associations evolved directly from non-anemone-associated ancestors in both facultative and obligate clades and that this behaviour has been lost on at least 2 occasions among facultative lineages (Fig. 2D). The same number of transitions was recorded when species with an unknown life-history stage of anemone association (18 species) were coded as “unknown” rather than “facultative”. Further, when we allowed the stochastic mapping analysis to predict whether unknown facultative taxa were mutualists as juveniles only or as juveniles and adults (i.e. facultative), it definitively predicted a tip to be facultative in only one instance (Moluccan cardinal fish *Ostorhinchus moluccensis –* 97% of stochastic maps). For all other unknown taxa, the prediction was equivocal (< 60% of maps in either stage) due to the lack of closely related taxa that are also associated with anemones. Given the lack of predictability and the distribution of body sizes in the unknown facultative taxa, we proceeded with analyses where unknowns were coded as “facultative”.

Using published records of the life stage(s) at which fishes associate with anemones (table S1), we tested whether the adult body size of fishes that associate with anemones correlates with the life history stage at which they maintain a mutualism. There were significant differences in maximum body size among mutualist categories (*F*3,1902 = 3.62, *P =* 0.012). We found that, within families that included both anemone-associated and non-anemone-associated species, lineages that have evolved a facultative association as juveniles-only (332 ± 34.8 mm) were significantly larger than non-mutualist lineages (239 ± 6.63 mm) (coefficient: 0.06, *t*1902 = 2.9*,* *P* = 0.003), while lineages that display facultative associations as both juveniles and adults (96.6 ± 6.86 mm) were not significantly smaller than non-mutualist lineages (coefficient: -0.02, *t*1902 = -1.35, *P* = 0.17), and obligately associated lineages (126 ± 4.87 mm) were also not significantly different in size from non-mutualists (coefficient: 0.05, *t*1902 = 0.75, *P* = 0.45) (Fig. 2E). These results did not change when we excluded the obligate anemonesfishes (*F*2, 1874 = 6.17, *P* = 0.002) from our analyses, when species with an unknown life-history stage of anemone association were excluded from our analysis (*F*3, 1884 = 4.17, *P* = 0.006), or when we removed the 18 mutualist species that were grafted to the molecular phylogeny (*F*3, 1884 = 6.08, *P* = 0.0004).

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

Our transect data revealed that there was a significant interaction between substrate type and fish size (, Fig. 3): whilst recruit (< 2 cm TL, *n* = 326) and juvenile (2 – 4 cm TL, *n* = 387) dascyllus, which were both found almost exclusively in association with anemones, did not differ in their substrate use (coefficient: 1.35, *z* = 1.24, *P* = 0.22), there was a progressive decrease in the use of the anemone in the larger dascyllus size classes (small adults, 4 – 6 cm TL, *n* = 240; coefficient: 3.39, *z* = 3.32, *P* = 0.0009; and large adults, > 6 cm TL, *n* = 169, coefficient: 6.60, *z* = 1.01, *P* < 0.0001). Our analysis also revealed an interaction between the abundance of fishes within a particular size class and anemone size (). However, with the exception of juvenile fishes, which were found in significantly higher abundances on larger anemones (coefficient: 0.23*,* *z* = 4.24, *P* < 0.0001), there were no significant interactions between fish size and anemone size in small adults (coefficient: 0.09*,* *z* = 1.37, *P* = 0.72) or large adults (coefficient: 0.04*,* *z* = 0.21, *P* = 0.84), indicating no evidence of larger fishes associating more frequently with larger anemones.

When investigating whether three different predators (arc-eye hawkfish, *n* = 10; speckled sandperch, *n* = 10; and smallmouth squirrelfish, *n =* 10) preferentially targeted small, medium or large dascyllus, we found no difference in prey body size preference ( = 57.46, *P* = 0.89). Across these three predators, recruit fishes were removed significantly more than both juvenile and small adult dascyllus by all three predator species ( = 57.46, *P* < 0.001) (Fig. 4a, b, c). When ten dascyllus and ten surge damselfish recruits were placed in a tank with an anemone, a coral, coral rubble, and a hawkfish predator (*n* = 20 trials), significantly more dascyllus survived compared to surge damselfishes (*F*1, 39 = 28.9, *P* < 0.0001, Fig. 4d). When ten dascyllus recruits were placed in a tank with coral rubble, a hawkfish predator and either a coral (*n* = 20 trials) or an anemone (*n* = 20 trials), significantly more dascyllus survived when an anemone refuge was available (*F*1, 39 = 31.98, *P* < 0.0001, Fig. 4e). We also detected no significant differences when we compared survival of dascyllus with access to a coral refuge to that of surge damselfishes (*W* = 161, *P* = 0.276), nor in total predation across these trials (*W* = 513.5, *P* = 0.063).

**DISCUSSION**

Predation has been shown to select protective mutualisms in a variety of species-specific case-studies (Bronstein 1994; Hay *et al.* 2004; Bascompte & Jordano 2007), as well as regulate mutualism dynamics between interacting species (Palmer *et al.* 2008; Canestrari *et al.* 2014). However despite being an intuitive expectation, no prior study appears to have directly investigated whether predation might also select globally generalizable patterns of convergence in interspecies mutualisms (Doebeli & Knowlton 1998; Toby Kiers *et al.* 2010; Bittleston *et al.* 2016). When considering fishes that form both facultative and obligate mutualisms with anemones, our phylogenetic and transition-state analyses indicate that: i) these relationships have evolved on numerous occasions, ii) while obligate associations with anemones evolved on one occasion, facultative associations have continuously emerged and disappeared over the past 60 MY, and iii) maximum adult body size is associated with the life-history stage at which fishes have been recorded to associate with anemones, with smaller-bodied species, or individuals within species, associating with anemones more than their larger counterparts. To investigate the causality of these patterns, we conducted field transects and a series of predation experiments on the threespot dascyllus as a case study, which show that: i) there is an inverse relationship between fish body size and its likelihood of being observed in association with an anemone, and no evidence that larger fishes seek out larger anemones, ii) three common piscivorous predators all preferentially target smaller prey over larger prey, iii) predators preferentially target prey from species that do not associate with anemones over those that do, and iv) within a species, individuals that associate with anemones are less likely to be preyed on compared to those that only have access to a coral refuge. Considering that several other species-specific case studies have suggested that protection from predators is a benefit of these relationships in both facultative (Elliott 1992) and obligate (Mariscal 1970b) fish lineages, our results strongly suggest that selection by predators has been instrumental in the recurrent convergent evolution of fish-anemone mutualisms, and that this pattern of evolutionary convergence is globally generalizable.

Our results highlight that fish-anemone mutualisms are geographically and phylogenetically common relationships. When considering facultative and obligate mutualisms together, our results show that these relationships have evolved on at least 55 occasions across 16 families (Fig. 1), indicating that over a quarter (27%) of coral reef associated fish families contain at least one representative that has been recorded to form mutualisms with anemones. While it is difficult to compare facultative versus obligate mutualisms, given that obligate relationships are only represented in the monophyletic anemonefishes, there does appear to be some notable differences between the two. Facultative mutualisms appear to be highly labile, behaviourally opportunistic relationships that have evolved on numerous occasions in geographically disparate locations, such as the Mediterranean, West-Atlantic, and Indo-Pacific. Once they emerge, they show little evidence of subsequent diversification, with exception of some small clades within the Pomacentridae, Apogonidae and Labrisomidae (Fig. 1). Obligate mutualisms, in contrast, are associated with accompanying specialized phenotypes, which may contribute to them being rarer and less labile compared to facultative mutualisms (Santini & Polacco 2006). The emergence of obligate mutualisms with anemones also appears to have preceded a period of rapid diversification (Cowman & Bellwood 2011; Litsios *et al.* 2012). One question concerns why obligate mutualisms have evolved so rarely compared to their facultative counterparts. However, this result is perhaps unsurprising given that the creation of reef habitats throughout the Indo-Australian Archipelago during the Oligocene and Miocene underpinned high rates of cladogenesis and niche-specialization (Cowman & Bellwood 2011). For example, cleaning and corallivory are relatively common and geographically diverse life-history strategies in coral reef fishes, but similar to anemone mutualisms, obligate representatives are also primarily restricted to the Indo-Pacific and evolved around this period (Cowman & Bellwood 2011; Frédérich *et al.* 2013; Baliga & Law 2016). Overall, despite facultative and obligate mutualisms appearing to exhibit at least some differences, the general lack of a pattern in the temporal emergence of these relationships over the past 60 MY as well as their geographic and phylogenetic diversity suggests that a pervasive ecological pressure, rather than a discrete biological or geological event, may best explain the repeated evolution of this trait.

The high fitness costs that are experienced by smaller fishes, regardless of their phylogenetic position or life-history stage, makes fish-anemone mutualisms well-suited to explore the ecological pressures that can select the recurrent evolution of interspecies mutualisms. The life-history of many reef-associated fishes comprises a dispersive larval phase followed by more sedentary juvenile and adult phases. Larval fishes face a severe predation bottleneck as they return to the reef (Almany & Webster 2006), with a recent meta-analysis estimating an average daily mortality rate of approximately 30% of predator-naïve recruits per day across species during this time (Goatley & Bellwood 2016). While the likelihood of predation dramatically decreases as experience with predators and/or body-size increases (Munday & Jones 1998; Depczynski & Bellwood 2006; Mitchell *et al.* 2013; Goatley & Bellwood 2016), mortality risk still averages approximately 3-8% per day until total length reaches approximately 43 mm, after which it stabilizes around 0.2% per day (Goatley & Bellwood 2016). When comparing across species, our phylogenetic analysis shows that maximum adult body size varies significantly with the stage at which species maintain mutualisms with anemones: species that associate with anemones as both juveniles and adults tend to be smaller bodied species, and species that associate with anemones exclusively as juveniles are significantly larger than non-mutualists as adults (Fig. 2E). Our field transects and aquaria experiments corroborate these patterns, showing that smaller fishes associate more with anemones compared to larger fishes, and that they are the primary beneficiaries of these mutualisms. It is interesting that our transect data, as well as data from other mutualism systems, indicates that these relationships weaken once individuals reach approximately 40 mm in length. For example, several species of tubelip wrasse (*Diproctacanthurus xanthurus* and *Labropsis alleni*) have been observed to exhibit cleaning behaviours, which are associated with decreased predation risk (Côté 2000) until they reach approximately 35-50 mm in length (Cole 2010; Grutter & Feeney 2016) and similar to fish-anemone associations, numerous small fishes obtain protective benefits through associations with other sessile organisms, such as corals or urchins, to whom they provide nutrition through their excrement (Liberman *et al.* 1995; Vagelli & Erdmann 2002). Therefore, while our study focused on fish-anemone mutualisms, our results may indicate that the gape-limitations inherent to many predatory fishes might help explain and unify diverse studies regarding the evolution of size-dependent protective mutualisms, especially in marine environments.

Our key insight is that, in addition to affecting mutualism evolution in species-specific case studies and regulating mutualism dynamics between interacting species (Palmer *et al.* 2008; Canestrari *et al.* 2014), the pressure imposed on prey by predators might also explain global patterns of convergence of interspecies mutualism evolution. While protective mutualisms are near ubiquitous components of almost all ecosystems (Bronstein 1994; Hay *et al.* 2004; Bascompte & Jordano 2007), the size-dependent nature of these relationships in marine environments appears to make them well-suited for discerning the pressures selecting protective mutualisms across a wide range of species compared to their terrestrial counterparts, where predation pressure is less linked to prey body size (Chamberlain & Holland 2009; Trager *et al.* 2010). We emphasise the importance of understanding how external pressures can influence convergent patterns of interspecies interactions and the fundamental role that predation has played in shaping the evolution of the world’s biodiversity.

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**Author Contributions** W.E.F and R.M.B conceived the study, with important contributions from A.M and P.F.C; phylogenetic comparative analyses and ancestral state reconstructions were conducted by P.F.C, with assistance from W.E.F, R.M.B, J.D.J.G and A.M; field and laboratory studies of fish behaviour were conducted by W.E.F and R.M.B, with assistance from 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 wrote the manuscript with input from all authors.

**Declaration of Interests** The authors declare no competing interests.

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**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).