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Substantial plasticity of reproduction and parental care in response to local resource availability in a wild clownfish population

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Plasticity, the capacity of individuals to respond to changing environments by modifying traits, may be critically important for population persistence by allowing for adaptive responses on shorter timescales than genetic change. Here, we use the clown anemonefish *Amphiprion percula*, whose access to resources is constrained by their anemones, to test the role of plasticity in generating variation in reproductive success among groups. We surveyed a wild clownfish population and found positive correlations between anemone area, fish size, reproduction and parental care. We used structural equation modeling to test the hypothesis that these correlations emanate from variation in anemone area and found support for a pathway linking anemone area to female investment, female investment to male investment and male investment to embryo survival. Next, we experimentally tested whether plasticity in response to resource availability can result in variation in parental traits using a feeding manipulation and found substantial plasticity in reproduction and parental care in response to changes in the availability of food resources. The results of this study reveal the role of plasticity in response to local resource availability in generating variation among individuals in reproductive strategies, linking studies of behavior and demography in this model species, and ultimately contributing to our ability to predict how populations might cope with environmental changes.

Keywords: *Amphiprion percula*, habitat quality, parental investment, plasticity, reproduction, resource availability

Introduction

Understanding how the environment influences reproductive success is critical to determining whether populations can persist in the face of environmental changes. One way that populations can respond to environmental variation is through local phenotypic adaptation, where the genotypes that perform best in the local environment

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are favored by selection (Hereford 2009, Villellas and García 2018). However, traits that influence reproductive success are predicted to have low additive genetic variances due to their association with fitness, and thus low capacity to evolve in response to selection (Fisher 1930, Lande 1982, Charmantier and Garant 2005). Another way that populations can respond to environmental variation is through phenotypic plasticity, the capacity of individuals to respond to changing environments by modifying physiological, morphological or behavioral traits. Plasticity may be particularly important to population persistence because plasticity is an organism's predicted primary response to environmental changes, allowing for biological innovation on shorter timescales than genetic change (West-Eberhard 2003). Furthermore, plasticity plays a key role in the evolutionary process by influencing which phenotypic traits are exposed to selection and promoting population persistence in variable environments (Pigliucci and Murren 2003, Ghalambor et al. 2007, Wund 2012). However, plasticity might not always be adaptive, for example in novel environments where the phenotypic response has not previously been exposed to selection (West-Eberhard 2003, Ghalambor et al. 2007, Gibert et al. 2019). Therefore, studying the role of plasticity in generating variation in fitness-associated traits is key to our ability to make predictions about whether and how populations will respond to variable environments.

Plasticity is predicted to be favored by selection when environments are spatially or temporally variable if it allows organisms to optimize their phenotypic expression of traits across environments (Dewitt et al. 1998, West-Eberhard 2003, Acasuso-Rivero et al. 2019). For example, when resources are temporally variable, an individual might benefit from plasticity by investing less in reproduction when resources are scarce compared to when resources are abundant (Trivers 1972, Stearns 1989, Reznick et al. 2000). Analogously, when resources are spatially variable, an individual might benefit from plasticity by investing less in reproduction when residing in a poor patch compared to a good patch. Indeed, differences among individuals or groups in the availability of local resources can result in positive correlations within individuals in fitness-associated traits such as size, growth and reproductive output (van Noordwijk and de Jong 1986, Stearns 1989, Reznick et al. 2000, West-Eberhard 2003). Furthermore, in species with biparental care, the social environment created by interactions between parents may play a role in generating variation in fitness-associated traits because a parent's optimal reproductive strategy depends not only on its own condition and access to resources, but on the condition and reproductive strategy of its partner (Head et al. 2012, Royle et al. 2014). The outcome of these interactions will determine how many offspring are produced as well as the amount of care those offspring receive, and therefore may have important consequences for population stability and persistence.

The clown anemonefish, *Amphiprion percula*, provides a model system in which to investigate questions about the consequences of plasticity in reproductive strategies, due to their close association with sea anemones, which provide access to resources, oviposition sites and protection from predators (Allen 1972, Fautin 1992, Feeney et al. 2019). Within each anemone, *A. percula* form social groups with a single, monogamous breeding pair (Buston 2004a, b). The breeding pair lay a clutch of approximately 324 ± 153 (mean $\pm$ SD) eggs, 0–3 times per lunar month (Buston and Elith 2011, Seymour et al. 2018), thus providing an extended context in which to study how parents respond to their environment. When a clutch is laid, both parents spend time tending to their eggs by cleaning, oxygenating and defending the nest for seven days until hatching (Buston and Elith 2011, Barbasch and Buston 2018). Observations have revealed robust positive associations between anemone size, female size and group size, suggesting that anemone size is an indicator of habitat quality (Fautin 1992, Elliott and Mariscal 2001, Buston and Elith 2011, Chausson et al. 2018). The positive associations between anemone size and female size are likely driven in part by the greater foraging area that larger anemones provide (Fautin 1992, Buston and Elith 2011, Verde et al. 2015), as the fish rarely venture more than a few centimeters beyond the perimeter of the anemone tentacles (Elliott and Mariscal 2001, Buston 2003b). Differences among anemones in size and thus the access to food and other resources they provide may therefore play a crucial role in generating variation in reproductive success among groups through its effects on female and male investment in growth, reproduction and parental care. However, no studies have experimentally tested whether plasticity of reproduction and parental care in response to local resource variation can result in among-group variation in reproductive success (Buston and Elith 2011, Salles et al. 2019).

This study tested how differences in long-term access to anemone resources and short-term changes in food availability influence fitness-associated traits and, ultimately, generate variation among groups in reproductive success. First, we monitored a wild population of anemonefish to test the hypothesis that size, parental investment and embryo survival will be positively correlated within groups due to differences in anemone area and thus differences in access to resources. Second, we tested whether there is support for a causal pathway linking anemone area to female and male size, fish size to reproduction, reproduction to parental care, and ultimately, parental care to embryo survival. Third, we used a feeding manipulation to experimentally test the hypothesis that parents respond to short-term increases in food resource availability by increasing reproductive output and parental care, which will improve the survival probability of their embryos. Taken together, these components reveal the role of variation among groups in habitat quality and the availability of local resources in generating variation among individuals in reproductive strategies, linking studies of behavior and demography in this model species, and ultimately contributing to our ability to predict how populations might cope with environmental changes.

Material and methods

Study population

Fieldwork was conducted on ten discrete platform reefs near Mahonia Na Dari Research and Education Center in Kimbe Bay, Papua New Guinea (5°16'66.667''S 150°5'E) using SCUBA. These nearshore reefs were located approximately 0.2–1.4 km from the shoreline and support a population of over 100 groups of *Amphipirion percula* living within magnificent sea anemones, *Heteractis magnifica* (Chausson et al. 2018, Rueger et al. 2018). In the two weeks prior to the start of the experiment, 117 groups were located and marked with numbered tags for identification. From 26 May to 23 June 2017, each of these 117 groups was visited once every two days and the reproduction and parental behaviors of the breeding pairs were monitored.

Groups consisted of up to five resident fish (female, male and 0–3 subordinates) with an average of 2.9 (± 0.1 SE) fish across all groups. Group size has been shown to be unrelated to reproductive success of the breeding pair, using long-term field observations in conjunction with experimental removal of non-breeders, analyzed using both mixed model analyses and boosted regression trees (Buston 2004a, b, Buston and Elith 2011). For this reason, group size was not included in our analyses.

Of the 117 total groups, 52 (44%) were observed with eggs at least once in the lunar month before the manipulation began and these were considered breeding groups. The 52 breeding groups were distributed across 10 reefs, five of which were surveyed each day, such that all 52 groups were surveyed every other day throughout the study. The experimental manipulation was conducted on these 52 breeding groups for two lunar months from 24 June to 21 August 2017. Lunar months were used rather than calendar months because pairs breed on lunar, semi-lunar or trient-lunar schedules (Buston and Elith 2011, Seymour et al. 2018).

This study began after a severe mass bleaching event that was observed from March to May in 2017 and n = 10 of our experimental anemones exhibited partial bleaching (<75% bleached). Anemone bleaching is known to influence anemonefish metabolism and reproduction (Beldade et al. 2017, Norin et al. 2018). We thus considered whether or not the anemone exhibited some bleaching when stratifying our treatments (see Experimental manipulation of resource availability).

Anemone area

Anemone area was used as a proxy of habitat quality. To measure anemone area, the length and width of the tentacle crown of each anemone was measured three times per month to the nearest cm using tailor's tape, such that the limits of the length and width measurements were the tips of the tentacles. The median of the three measurements was taken to account for the expansion behavior of anemone tentacles, and the area of each anemone was estimated using the equation for the area of an ellipse (Hattori 1995, Mitchell 2003): Area = $\pi \times ab$, where $a = \frac{1}{2}$ the length and $b = \frac{1}{2}$ the width. These monthly median area estimates were then averaged for each anemone to provide a single anemone area measurement.

Body size

To determine if there are positive correlations between anemone area and parental body size, female standard length (FSL) and male standard length (MSL) were measured. The female and male from each group were captured using hand nets in the week before the start of the experiment (week of 26 May 2017) and in the week following the end of the experiment (week of 21 August 2017). Each fish was photographed to identify individuals by their color patterns and to confirm that they survived and did not move between anemones (Nelson et al. 1998, Elliott and Mariscal 2001, Buston 2003a). The standard length (SL) of each fish was measured to the nearest 0.1 mm using calipers, and the average of the two measurements (start and end) provided a single measurement of FSL and MSL. The change in SL between the start and end measurements was small, with an average increase of 3.2 mm (± 1.9 SD) for females and 2.5 mm (± 2.4 SD) for males.

Reproductive success

To determine if there are positive correlations between anemone area, body size and reproductive output, reproduction was monitored for all 52 groups every other day. Reproduction was detectable by the behavior of the male and the presence of eggs at the base of the anemone. The exact age of the eggs was determined by their color: day 1 = orange, day 2 = orange-brown, day 3 = brown, day 4 = black eyes, day 5 = silver eyes, day 6 = silver eyes with pupils, day 7 = gold eyes with pupils (Buston 2004a). Eggs hatched after seven days. Photos were taken of every clutch observed with a camera early (day 1 or 2) and late (day 6 or 7) in development. The number of eggs per photograph was counted using ImageJ (NIH, USA). Reproductive success was quantified by the number of eggs laid per clutch and embryo survival (the proportion of eggs laid that survived to day of hatching). The number of eggs laid per clutch and embryo survival were chosen as measures of reproductive success, as the number of eggs produced has been shown to be related to the number of recruits produced in other anemonefishes (Saenz-Agudelo et al. 2015).

Parental care

To determine whether parental care was positively correlated with anemone area, other parental traits and reproductive success, 29 min videos were taken of parental behavior. Because parental behavior has been shown to increase as the clutch develops, but no significant increase in tending was observed from day 1 to day 4 (Barbasch and Buston 2018), videos were taken early (day 3 or 4) and late (day 6 or 7) in development by setting up a tripod with an underwater camera centered

on the location of the eggs. Videos were analyzed using JWatcher ver. 0.9 (Blumstein and Daniel 2007). The first 12 min of video served as an acclimation period, the following 15 min used for data collection and the final 2 min were discarded from analysis to minimize any effect of approaching researchers at the end of the recordings. A laboratory study in *A. percula* demonstrated that individual parental behaviors are consistent between clutches and that 15 min was a sufficient period of time to observe variation within and among individuals in parenting behavior (Barbasch and Buston 2018). Parental care was recorded as tending, i.e. the amount of time an individual spent within one body-length of the clutch, as tending behavior shows plasticity in both parents (Barbasch and Buston 2018). Behavior was scored separately for the male and female of each pair and blind to feeding treatments.

Experimental manipulation of resource availability

To determine whether variation in the availability of local resources generates positive correlations between parental traits, feeding manipulations were conducted. One lunar month of baseline data was collected (26 May–23 June 2017) on 117 groups, followed by two lunar months of feeding manipulation (24 June–21 August 2017) on 52 breeding groups. Of the 52 breeding groups, 25 were given supplemental food and 27 served as controls. As reefs were located at different distances from shore, and a few anemones exhibited a small amount of bleaching, groups were randomly stratified by location for reefs closer to shore (n = 32 anemones) versus further from shore (n = 20 anemones) and for bleached (n = 10) versus unbleached (n = 42) anemones (Table 1). Average female size was similar in fed (average SL = 52.8 mm ± 0.87 SE) and control groups (average SL = 52.3 ± 1.12 SE), and the number of clutches laid by pairs in the first lunar month was similar in fed (once: n = 14; twice: n = 11) and control (once: n = 13, twice: n = 14) groups to control for differences among pairs in reproductive strategy (Seymour et al. 2018).

Fed groups were supplemented with 3 ml freeze dried brine shrimp and 3 ml fish pellets. Food was kept dry in capped tubes until delivery. Brine shrimp (positively buoyant) were

delivered first, by squirting the shrimp just below the anemone with a pipette. Pellets (negatively buoyant) were delivered second by opening the vial and tipping them down onto the anemone. Through this method, most food was either immediately consumed by the fish or stuck among anemone tentacles, where the fish could consume it. Some food was consumed by the anemone or other fish species nearby. Confirmation of successful administration of food was made by observing the male and female of each group consuming at least one pellet or shrimp. All experimental groups were supplemented with food every other day (each time they were visited) for the two lunar months following the start of the manipulation, excepting the days when videos were taken to minimize the disturbance to the pair during the recording. Thus, supplemented groups were fed approximately 28 times during the manipulation. Control groups received a sham feeding treatment, using an empty vial and an empty pipette, to keep disturbance to all groups consistent. We were thus able to quantify plasticity in response to food availability by comparing changes in reproduction and parental care pre- and post-manipulation in the fed groups relative to the control groups.

Statistical analyses

Multivariate model of habitat quality and parental traits

To examine correlations between anemone area and parental traits (body size, reproductive success and parental care), a multivariate generalized linear mixed model was fit in a Bayesian framework using the *MCMCglmm* package (Hadfield 2010) in R ver. 3.6.3 (<www.r-project.org>). This modeling framework allowed us to examine the effect of food availability on multiple parental response variables and to examine the variance–covariance matrix (Houslay and Wilson 2017) to calculate the correlations within groups among anemone area, an indicator of habitat quality and parental traits.

To determine pairwise correlations among traits, anemone area and six parental traits were fit as response variables: female standard length (FSL), male standard length (MSL), male tending, female tending, number of eggs laid per clutch and embryo survival. Anemone area, FSL, MSL and male and female tending were fit with Gaussian error distributions. Number of eggs laid was fit with a Poisson error distribution. Embryo survival was fit with a binomial distribution. To control for repeated measures within groups, group ID was fit as a random effect.

Feeding treatment (Fed or Control), month (1: pre-treatment, 3: post-treatment), and the interaction between treatment and month were fit as predictor variables. Month 1 was pre-treatment and month 3 was post-treatment, therefore the interaction between treatment and month gives an estimate of the effect of feeding treatment on parental traits. (Data from month 2 were not included in the analysis, to allow one month for the treatment to take effect.) Clutch age was fit as a 4-level categorical predictor for male and female tending (day 3, 4, 6 and 7).

Table 1. Outcome of the random stratification of anemone groups from each treatment (Fed or Control). Groups were stratified by whether or not they exhibited bleaching (bleaching status: unbleached or bleached), whether located on a reef closer to shore or farther from shore (reef location: inshore or offshore), and the number of times the pair laid a clutch of eggs in the first lunar month prior to the start of the manipulation (clutches: one or two). Anemones within each subgroup were then randomly assigned a treatment (Fed or Control).

Treatment	Bleaching status	Reef location	Clutches
Fed	20 unbleached, 5 bleached	13 inshore, 14 offshore	14 one, 11 two
Control	22 unbleached, 5 bleached	11 inshore, 14 offshore	13 one, 14 two

Normal priors were used for fixed effects, which assume a normally distributed posterior and use a mean of zero with a large variance (Hadfield 2010). Priors for residual variance of male and female tending, number of eggs laid and embryo survival were set as the inverse gamma distribution (shape = scale = nu/2; nu = 1.002, V = 1 in the MCMCglmm notation), which are expected to be weakly informative priors (Hadfield 2010), and flat parameter-expanded priors were set for the random variance (V = 1, nu = 1; prior mean: alpha. mu = 0, covariance: alpha.V = 25²), which improve convergence in MCMC models (Gelman 2006). The residual variances for FSL, MSL and anemone area were fixed at a small value (0.01) since these measurements were averaged across the study period and thus had no within-individual variation, and model results were robust to choice of scale parameter for residual variance or use of inverse-Wishart rather than inverse gamma priors for residual variances. The model was run for 5 040 000 iterations with a burn-in of 40 000 and chains were thinned by 1000, giving a total number of MCMC samples of 5000. These specifications were chosen because they resulted in model convergence and low autocorrelation, which were confirmed through visual inspection of the traces, density plots and autocorrelation plots. The burn-in of 40 000 was used to allow the chain to reach its equilibrium, and thinning of 1000 was used to improve chain mixing.

Structural equation model of hypothesized causal pathways

To test a series of hypothesized causal pathways linking anemone area with parental investment and reproductive success, we used piecewise structural equation modeling (SEM). This statistical technique allows for the simultaneous test of multiple causal hypotheses and estimates the strengths of the effects using the linear relationships between variables, while taking into account non-gaussian error distributions and repeated measures (Shipley 2000).

The causal directions between variables were inferred based on previous literature. Anemone area was hypothesized to influence female size due to large anemones providing access to more resources, causing increased growth and resulting in larger females (Fautin 1992, Elliott and Mariscal 2001, Buston 2002, Chausson et al. 2018). Female standard length (FSL) was hypothesized to influence male standard length (MSL) due to the rules of the size-based dominance hierarchy in *A. percula* (Buston 2003a, Buston and Cant 2006, Chausson et al. 2018). FSL and MSL were hypothesized to influence egg production and parental behavior, which in turn may influence embryo survival (Buston and Elith 2011). Additionally, correlated errors were fit between male and female tending, as there is no support for a causal, unidirectional relationship between male and female behavior (Barbasch and Buston 2018).

To fit our models, we used the *piecewiseSEM* package in R (Shipley 2009). We fit eight response variables and performed forward model selection using χ² difference tests to compare nested models as each pathway was added (Shipley 2000). First, we fit our habitat quality indicator: anemone area. Then we fit the parental traits hypothesized to be influenced by anemone area: FSL and MSL. Finally, we fit four reproductive and parental care traits hypothesized to be influenced by resource availability: number of eggs laid, male tending, female tending and embryo survival. For number of eggs laid, male tending, female tending and embryo survival, a random effect group ID was included in the models to account for repeated measures within groups. Furthermore, male and female tending were restricted to late in egg development (day 6 or 7) because most of the behavioral variation is seen at later developmental stages (Barbasch and Buston 2018). FSL, MSL and male and female tending were fit with Gaussian error distributions while eggs laid and embryo survival were fit with Poisson and binomial error distributions, respectively.

Our model selection allowed us to test hypotheses about the indirect downstream effects of anemone area, FSL and MSL by comparing the fit of models with and without each possible direct link between these metrics and number of eggs laid, male and female tending and embryo survival. Specifically, our null model contained anemone area as a predictor of FSL, and FSL as a predictor of MSL, and variables number of eggs laid, female tending, male tending and embryo survival were added sequentially. Nested models including and excluding each possible pathway between variables were compared using chi-squared difference tests. For MSL, we tested whether adding anemone area as a predictor significantly improved model fit. For number of eggs laid, we tested whether each sequential addition of MSL, FSL and anemone area improved model fit. For female and male tending, we tested whether sequential addition of number of eggs laid, MSL, FSL and anemone area as predictors significantly improved model fit. Finally, for embryo survival, we tested whether including female tending, male tending, number of eggs laid, MSL, FSL and anemone area as predictors significantly improved model fit. The final model was chosen using parsimony to include only the predictors that significantly improved model fit.

Results

Multivariate model of habitat quality and parental traits

First, we tested the hypothesis that natural variation in anemone area results in positive correlations between parental traits. As predicted, anemone area was positively correlated with FSL (correlation = 0.77, 95% credible interval (CI) = 0.67–0.86), MSL (correlation = 0.64, 95% CI = 0.50–0.78), number of eggs laid (correlation = 0.50, 95% CI = 0.31–0.70) and male tending (correlation = 0.63, 95% CI = 0.41–0.85) (Fig. 1). Anemone area was not correlated with female tending or embryo survival (Fig. 1).

Further, as predicted, positive correlations existed between the parental traits themselves. FSL and MSL were positively correlated with each other (correlation = 0.72, 95% CI = 0.60–0.83). FSL was positively correlated with number of eggs laid (correlation = 0.65, 95% CI = 0.48–0.80)
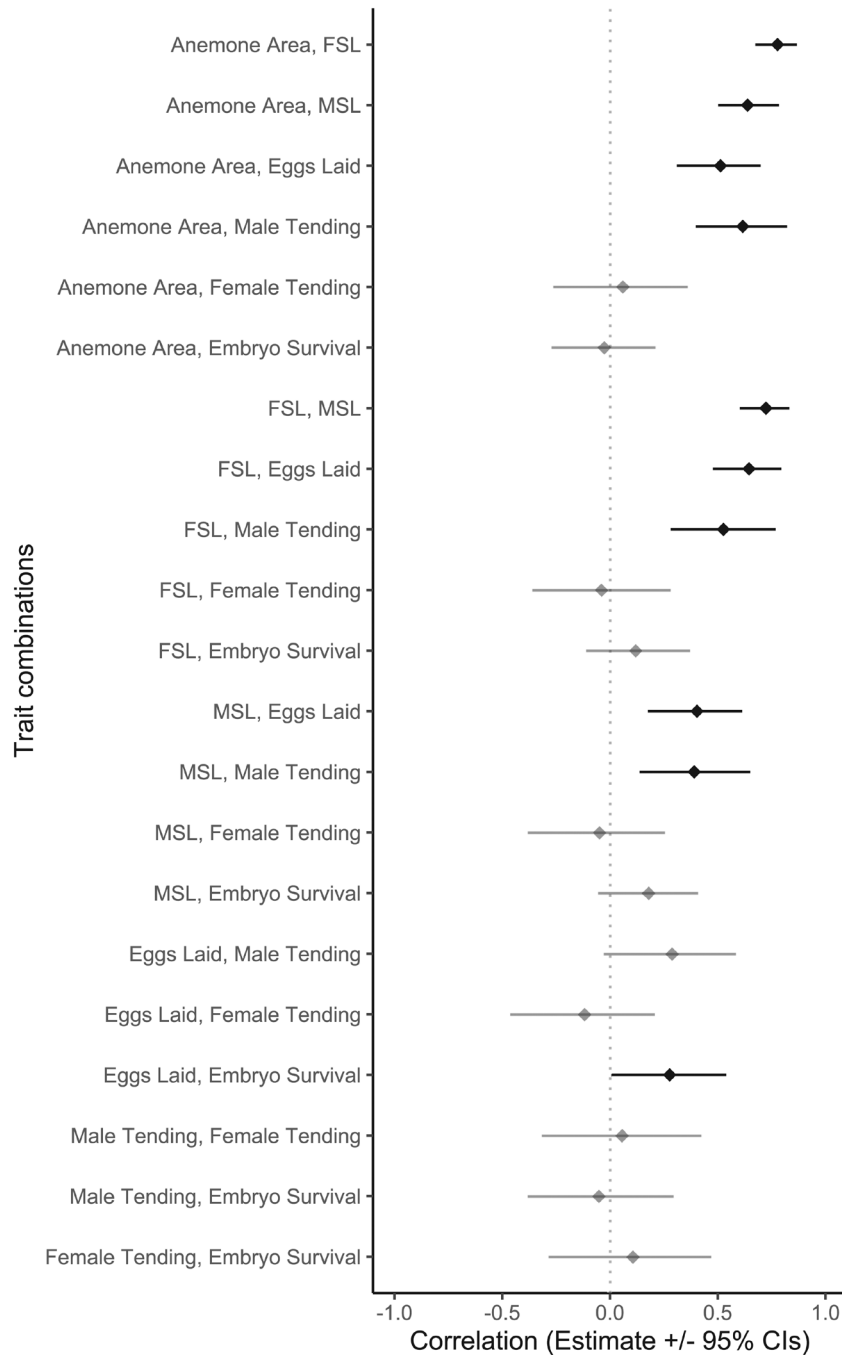
Figure 1. Correlations and 95% credible intervals for all pairwise combinations of habitat quality and parental traits. Points represent correlations between traits and lines represent 95% credible intervals around the correlation estimates from the covariance matrix of response variables from the MCMCglmm model. Significant correlations are bolded. The model was fit with seven response variables that fall into three categories: habitat quality (anemone area); relatively unresponsive parental investment traits (FSL and MSL); relatively responsive parental investment traits (number of eggs laid, male tending, female tending and embryo survival).

and male tending (correlation = 0.53, 95% CI = 0.28–0.77). MSL was positively correlated with number of eggs laid (correlation = 0.40, 95% CI = 0.18–0.61) and male tending (correlation = 0.39, 95% CI = 0.14–0.65). Finally, the number of eggs laid was positively correlated with embryo survival (correlation = 0.27, 95% CI = 0.01–0.54) (Fig. 1).

Structural equation model of hypothesized causal pathways

We tested the potential causal pathways explaining the correlations between anemone area and parental traits. The final SEM reveals the strengths of the pathways linking anemone
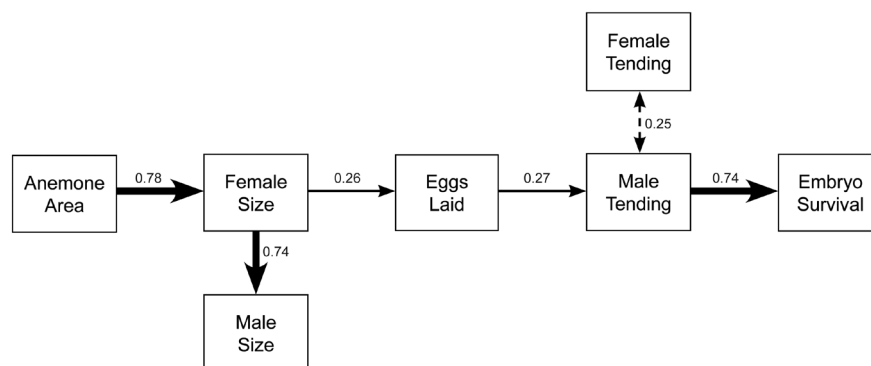
Figure 2. Structural equation model of the effect of habitat quality on male and female investment and reproductive success. Significant direct effects are indicated by arrows, weighted by the strengths of the standardized coefficients for each pathway, and the corresponding numbers indicate the standardized regression coefficient for each pathway. The double-headed dashed arrow corresponds to the correlated error fit between male and female tending.

area, parental size, egg production, parental care and embryo survival (Fig. 2). The overall model fit was assessed using AIC and Fisher's C statistic (AIC = 71.64; Fisher's C = 21.64, df = 18, p-value = 0.25). Note that a p-value greater than 0.05 indicates that the hypothesized structure is supported by the data. Model fit was also assessed using the individual R^2 values (FSL: marginal R^2 = 0.60; MSL: marginal R^2 = 0.58; Eggs laid: marginal R^2 = 0.41, conditional R^2 = 0.99; Male tending: marginal R^2 = 0.18, conditional R^2 = 0.56; Female tending: marginal R^2 = 0.02; conditional R^2 = 0.54; Embryo survival: marginal R^2 = 0.11, conditional R^2 = 0.11). Note that the marginal R^2 takes into account only the variance of the fixed effects, while conditional R^2 takes into account the variance of both the fixed and random effects (Nakagawa et al. 2017). Standardized coefficient estimates indicate the relative strengths of all direct effects of one variable on another, while the p-value indicates whether that effect was significant, controlling for the effects of the other predictors.

In the final model, which was the best fitting model based on the χ^2 difference tests comparing nested models, there was support for a pathway linking anemone area to female size, female size to reproductive output, reproductive output to male parental care and male care to embryo survival (Fig. 2). Anemone area was a significant predictor of FSL (std. estimate = 0.77, df = 72, p-value < 0.001). FSL in turn had a significant positive effect on MSL (std. estimate = 0.74, df = 72, p-value < 0.001) and number of eggs laid (std. estimate = 0.26, df = 118, p-value = 0.015). Number of eggs laid had a significant positive effect on male tending (std. estimate = 0.27, df = 89, p-value = 0.003), and male tending had a significant positive effect on embryo survival (std. estimate = 0.74, df = 115, p-value = 0.001). The correlated error between male and female tending late in egg development was significant (std. estimate = 0.25, df = 115, p-value = 0.003), indicating a significant positive correlation between female and male tending late in development due to randomness or some external factor influencing both parents' behaviors, rather than a causal relationship between them. This specification was based on our assumption that

there is no causal pathway between female and male tending, but that does not mean there could not be such a pathway. Nevertheless, there was no support for a pathway linking number of eggs laid to female tending or female tending to embryo survival, indicating that female tending had no significant effect on the causal pathway linking anemone area to embryo survival except through any potential indirect effects via male tending. None of the additional effects included in our final model were significant (i.e. anemone area on eggs laid or male tending; FSL on male tending; MSL on eggs laid or male tending; eggs laid on female tending), but these effects were included in the final model as including each one significantly improved model fit.

Experimental manipulation of resource availability

To experimentally test the hypothesis that plasticity in response to changes in resource availability generates variation in parental traits across groups (Fig. 2), we manipulated food availability. There were significant effects of month and clutch age (Table 1): the number of eggs laid and male tending were significantly greater in month 3 compared to month 1, and males and females spent significantly more time tending in late compared to early stages of egg development. There were also significant overall effects of treatment (Table 2). However, it is the interaction between treatment and month that reveals the effect of supplemental feeding, as month 1 was pre-manipulation and month 3 was post-manipulation.

There was a positive interaction between month and feeding for the number of eggs laid (Fig. 3a). The posterior mean for the interaction indicates that food-supplemented females increased the number of eggs laid from pre- to post-manipulation by 51 percent more than did control females (Treatment(F–C) × Month(post): posterior mean = 0.39; Table 1). There was also a significant interaction between treatment and month for male and female tending (Fig. 3b–c). The posterior means for the interactions indicate that fed males increase their tending from pre- to post-manipulation by 15 percent more than control males

Table 2. Summary of the results for the multivariate generalized linear mixed effect model (GLMM) testing the effect of food availability on multiple anemone and parental response variables. Results are shown for the effect of supplemental feeding on four response variables: number of eggs laid per clutch, female tending, male tending and embryo survival. The model includes the independent variables treatment (Fed: F; control: C), month (pre- and post-manipulation), clutch age (days 3, 4, 6, 7) and the interaction between month and treatment. Anemone ID was fit as a random effect. Shown are the fixed effects, posterior means, lower and upper 95% credible intervals, effective sample sizes and p-values. Effective sample sizes represent the number of samples drawn from the posterior distribution, adjusted for auto-correlation in the chains. Significant p-values are bolded.

Trait	Response variable	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
Eggs Laid	Treatment (F–C)	−0.40	−0.77	−0.07	5204	**0.0252**
	Month (post–pre)	0.50	0.37	0.65	5000	**<0.0002**
	Treatment (F–C) × Month (post–pre)	0.39	0.18	0.61	5000	**0.0004**
Female Tending	Treatment (F–C)	0.18	−0.81	1.19	5300	0.7436
	Month (post–pre)	0.12	−0.59	0.79	5000	0.7416
	Clutch Age (7–3)	0.68	0.11	1.25	5000	**0.0180**
	Clutch Age (7–4)	0.96	0.25	1.71	4233	**0.0112**
	Clutch Age (7–6)	0.40	−0.36	1.14	5000	0.2944
	Treatment (F–C) × Month (post–pre)	1.28	0.31	2.34	4803	**0.0132**
Male tending	Treatment (F–C)	−0.10	−1.63	1.53	4567	0.9008
	Month (post–pre)	1.17	0.23	2.14	5000	**0.0196**
	Clutch Age (7–3)	2.23	1.40	3.02	5000	**<0.0002**
	Clutch Age (7–4)	1.85	0.84	2.91	4647	**0.0004**
	Clutch Age (7–6)	1.01	**−0.02**	2.14	5000	0.0624
	Treatment (F–C) × Month (post–pre)	1.66	0.27	3.13	5000	**0.0272**
Embryo survival	Treatment (F–C)	0.59	−0.74	2.17	4440	0.4176
	Month (post–pre)	0.55	0.17	0.89	5000	**0.0040**
	Treatment (F–C) × Month (post–pre)	−0.32	−0.89	0.22	4647	0.2692

(Treatment(F–C) × Month(post): posterior mean = 1.66; Table 1), and fed females by 62 percent more than control females (Treatment(F–C) × Month(post): posterior mean = 1.28; Table 1). There was no significant interaction between treatment and month for embryo survival (Fig. 3d).

Discussion

Our results underscore the key role of the local environment in generating variation among individuals in reproduction and parental care. First, we found positive correlations between anemone area, parental size, reproductive output and parenting behavior suggesting that the quality of the anemone habitat may generate variation among groups in these traits. Next, we tested this hypothesis using structural equation modeling. We found support for a pathway linking anemone area and embryo survival through female and male investment in reproduction and parental care. Anemone area, a measure of habitat quality, had a direct effect on female size and consequently egg production, suggesting that females in good quality habitats grow larger and produce more eggs. Males provided more care to larger, potentially more valuable clutches, which improved embryo survival. Lastly, we experimentally tested the hypothesis that plasticity in response to resource availability causes variation among groups in reproductive strategy. We found that groups supplemented with food produce more eggs and provide more parental care than groups not supplemented with food, indicating that reproductive and parental traits are plastic in response to changes in resource availability. Together, these results demonstrate that plasticity in response to the local environment can result

in substantial variation among groups in reproductive output and parenting strategies, revealing the potential consequences of changes in environmental quality for populations.

Environmental variation can generate positive correlations between traits if it creates variation among individuals in their ability to invest in these traits (van Noordwijk and de Jong 1986, Reznick et al. 2000). Here, we found positive correlations between anemone area, female size and male size, which have been extensively documented in anemonefishes and are hypothesized to result from variation in patch quality (Allen 1972, Fricke 1979, Fautin 1992, Mitchell and Dill 2005, Buston and Elith 2011, Chausson et al. 2018). However, we found additional positive correlations between these and two parental traits (number of eggs laid and male parental care), providing novel support for the hypothesis that plasticity in female and male reproductive strategies in response to variation in habitat quality results in variation among groups in reproductive success.

Considering female investment, we found evidence that positive correlations between anemone area, female size and male size, and the number of eggs laid are best explained by the effect of anemone area on female size and the effect of female size on the number of eggs laid per clutch. Correlations between female size and egg production have been widely documented in fishes, and are explained by there being a physical constraint on the maximum number of eggs a female of a given size can produce (Trippel et al. 1997, Jennings et al. 2009, Saenz-Agudelo et al. 2015, Barneche et al. 2018). The results of our SEM similarly support a capacity constraint limiting egg production by size. However, we also found evidence that females with greater access to resources produce more eggs than females with less access to resources when
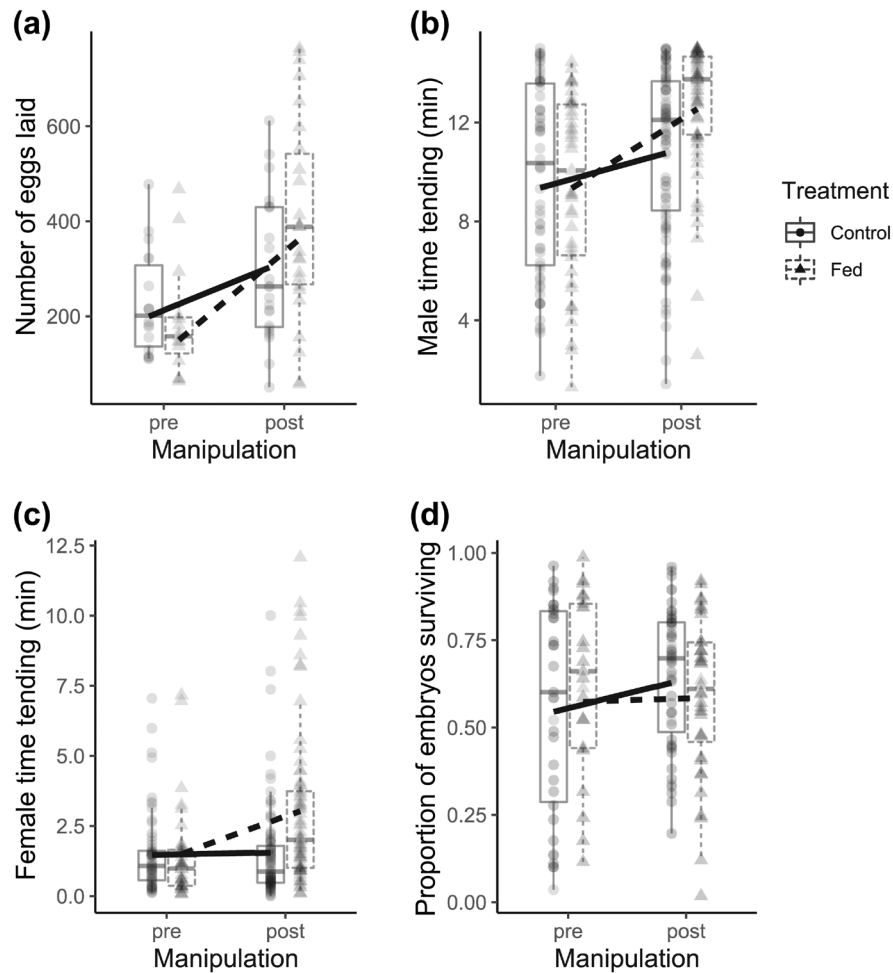
Figure 3. Effect of treatment on parental care and reproduction in the field. Points represent raw data, with control groups indicated by circles and fed groups indicated by triangles, and boxplots show the median and first and third quartiles for the control group (solid box) and fed group (dashed box). Fitted lines were generated using predicted values from MCMCglmm model fits for control (solid lines) and fed (dashed lines) groups for the effect of the interaction between treatment and month in the field on (a) number of eggs laid per clutch, (b) male tending (mins out of 15), (c) female tending (mins out of 15) and (d) Proportion of embryos that survived (embryo survival). Clutch age was fit as a covariate for male and female tending and group ID was fit as a random effect. Points were grouped horizontally by manipulation in all plots, as manipulation is discrete.

controlling for size. Together with the effect of anemone area on female size, these results suggest that females respond to resource variation in two ways on two timescales (Fig. 4): first, females in good habitats can grow more, become larger and thus have greater potential egg production because of their size; second, females in good habitats and with more resources invest more and have greater actual egg production for their size. These two types of responses may reflect female investment in capital (i.e. size) versus income (i.e. current food intake) (Stephens et al. 2009). Thus variation in resources can have both long-term (indirect via growth and size) and short-term (direct via egg production) effects on the number of eggs produced, generating extensive variation among females in reproductive output.

Considering male investment, we found that the positive correlations between anemone area, female size and male size, and the amount of time males spent tending are best

explained by the effect of anemone area on female size, the effect of female size on the number of eggs laid, and the effect of number of eggs laid on male tending. There are two, non-exclusive, explanations for this increase in male care when many eggs are present. First, males in good environments may spend more time tending because they have more energetic reserves to invest (van Noordwijk and de Jong 1986, Reznick et al. 2000). Second, males may allocate more to more valuable clutches and limit investment in, or even cannibalize, small clutches due to their low value in terms of lifetime reproductive success (Kvarnemo et al. 1998, Manica 2002, Klug and Bonsall 2007). Both explanations are consistent with our observational and experimental results, so we are unable to discriminate between the effects of food resource availability versus reproductive value on male investment. Further, we found that in groups with males that spent more time tending to the eggs at late (but not early) stages
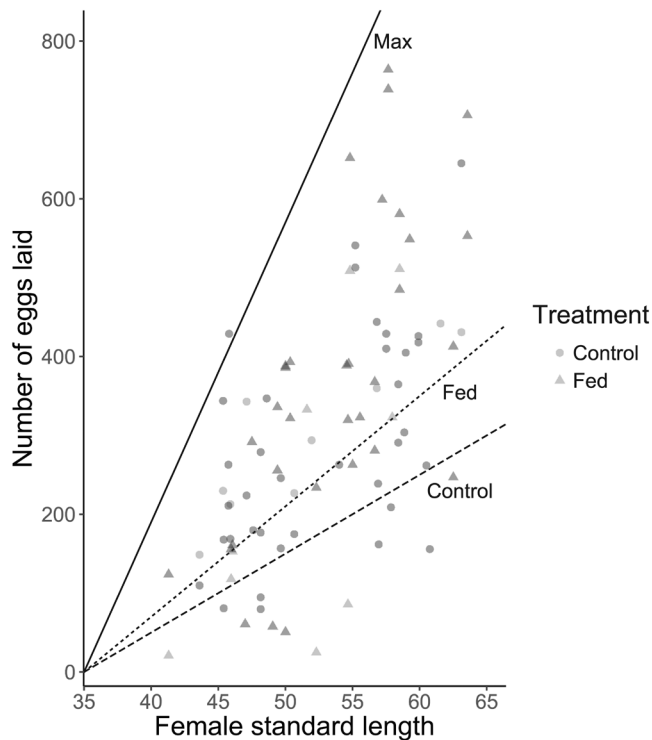
Figure 4. Conceptual model illustrating capital/long-term (standard length) and income/short-term (eggs laid) investments in lifetime reproduction by female clownfish. It is assumed that there is a minimum size at which females can reproduce (x-intercept: 35 mm; unpubl.). Females in large anemones, with access to large amounts of resources, will grow more than females in small anemones. This will be a long-term investment in reproduction, giving females greater potential egg production (upper, solid line). Females in large anemones, with access to large amounts of resources, will lay more eggs for their size (middle, dotted line) than females in small anemones (lower, dashed line). This is a short-term investment in reproduction, giving females greater actual egg production for their size. [Points show observed data from month 3 (post-manipulation) with circles representing control groups and triangles representing fed groups; lower-dashed and middle-dotted lines are best fit through the control group and fed group data respectively, assuming the minimum size of reproduction and controlling for group ID as a random effect; upper-solid line is best fit through the number of eggs laid by the three females with the highest reproductive output relative to size and the minimum size of reproduction.]

in development, a greater proportion of embryos survived to hatching. Together with the previous results, this suggests that mothers can influence the pair's reproductive success through potential and actual egg production and fathers can influence the pair's reproductive success through parental care.

A study by Head et al. (2014) conducted on biparental burying beetles similarly demonstrated that female and male investment strategies differed in how they influenced offspring performance. Similar to our study, the authors found that the duration of care provided by the primary caregiver (in burying beetles, the female), was the primary determinant of offspring performance, and that female investment in turn influenced male investment. In contrast to our study,

however, male care in burying beetles was negatively related to the duration of female care, suggesting that sexual conflict and social interactions between females and males may explain this variation in parenting strategies. In *Amphiprion percula*, females and males form long-term monogamous pairs and parents have few outside options as anemone habitat is saturated and the probability of predation outside the anemone is high (Buston 2003b), suggesting that unlike in burying beetles, sexual conflict might be low. Thus, in *A. percula*, females and males may be favored to coordinate their behaviors to reach a cooperative outcome that maximizes pair reproductive success. This might further help explain why pairs respond in similar ways to changes in resource availability, because by coordinating their responses, pairs may be able to optimize their reproductive allocation strategies by improving embryo survival through greater combined investment in reproduction and parental care when environmental conditions are good, while both limiting investment in reproduction and care when conditions are poor.

Our results, when interpreted alongside current literature, provide insight into how populations will respond to environmental changes. A study by Salles et al. (2019) found evidence for strong effects of habitat characteristics, notably anemone species and location, and weak genetic effects on the lifetime reproductive success of *A. percula*, and suggested that this species is particularly susceptible to changes in habitat quality. Troublingly, the low additive genetic variation Salles et al. (2019) found for reproductive success implies limited adaptive potential (Fisher 1930). In cases where adaptive potential is low, plasticity in fitness-associated traits may be of critical importance by allowing for rapid, population-level responses and persistence in the face of environmental changes (Pigliucci and Murren 2003, Ghalambor et al. 2007, Wund 2012). Furthermore, if there is genetic variation underlying plasticity, measured as genotype by environment interactions, then there is the potential for plasticity to evolve in response to selection (Sgrò and Hoffmann 2004, Nussey et al. 2007, Dingemanse and Wolf 2013, Adams et al. 2015). Our field survey found evidence for the key role of local environmental heterogeneity in generating variation in parental investment and reproductive output in *A. percula*, even within a single host anemone species. Furthermore, our experimental manipulation found evidence of substantial plasticity in reproduction and parental care in response to local resource availability, providing some hope for population stability and persistence despite environmental changes.

Conclusions

Our study highlights the vital role habitat quality plays in the stability and persistence of *A. percula* populations. We found that variation in access to resources among anemones of different sizes can generate variation among pairs of anemonefish in their reproductive strategies. This variation in turn has consequences for reproductive success. Furthermore, low additive genetic variance in reproductive success implies

limited adaptive potential in response to habitat degradation (Salles et al. 2019). Therefore, plasticity in reproduction and parental care may be of crucial importance. Our study provides evidence for extensive plasticity in reproduction and parental care, which may allow individuals to optimize their reproductive allocation in response to variable environments. An important next step in evaluating the evolutionary potential of this population is to determine whether or not plasticity is adaptive in this context. Genetic variation in the reaction norm would suggest that adaptive responses can evolve under selection (Nussey et al. 2007). Furthermore, individual variation in plasticity arising from past experiences can theoretically stabilize population-level responses, simply due to the diversity of responses in the population (West-Eberhard 2003, Wolf and Weissing 2010, Dingemanse and Wolf 2013). Therefore, even in the absence of genetic variation, individual variation might mitigate the negative effects of environmental changes. By allowing individuals to respond to changing environmental conditions, plasticity may provide a critical lifeline to populations.

Data availability statement

Data are available from the Biological and Chemical Oceanography Data Management Office: <www.bco-dmo.org/project/721455>.

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