Animal Behaviour 136 (2018) 65–73

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Plasticity and personality of parental care in the clown anemonefish

Tina A. Barbasch*, Peter M. Buston

Department of Biology and Marine Program, Boston University, Boston, MA, U.S.A.

ARTICLE INFO

Article history:
Received 21 December 2016
Initial acceptance 1 March 2017
Final acceptance 19 October 2017

MS. number: A16-01094R2

Keywords:
Amphiprion percula
individual variation
parental care
personality
plasticity
reaction norm
resource availability

Characterizing individual variation in parental care is critical to understanding how selection shapes and maintains patterns of care, yet little is known about how individual parents vary in their responses to the environment. Reaction norms, functions that describe how phenotypes change across an environmental gradient, provide an elegant framework for studying individual variation in behavioural responses. We use a reaction norm approach to investigate how studying plasticity, which describes variation within an individual through time, and personality, which describes repeatable variation among individuals, together explain individual variation in the parental behaviour of the anemonefish *Amphiprion percula*. More specifically, we test how resource availability influences individual parental responses to the environment and discuss the consequences for our understanding of plasticity and personality in parental care. Breeding pairs of *A. percula* were fed either a high or a low food ration and their parental behaviours were monitored. Individuals exhibited plasticity in parental behaviour across the two resource environments. Furthermore, individuals were repeatable in their behaviour through time, as evidenced by significant among-individual variation in intercept. Finally, the slope and elevation of individual reaction norms varied, revealing a level of variation not captured at the population level and providing insight into the potential mechanisms generating individual variation.

Parental care consists of an incredible diversity of forms. Patterns of care vary within individuals through time as well as among individuals in a population (Royle, Smiseth, & Kölliker, 2012). Explanations for variation in patterns of care stem from the theory that parents face a trade-off between investing in current versus future reproductive opportunities, and therefore the benefit of care to increased offspring fitness must be balanced against its cost to residual reproductive value (Trivers, 1972). Furthermore, an individual's optimal level of care can be influenced by genes, development, social and ecological context, or a combination thereof (Dingemanse & Wolf, 2013). Therefore, the costs and benefits of providing care at any point in time can be influenced by past, current and expected future experiences, and this explains why there might be so much variation in parental care (Dingemanse & Wolf, 2013; Royle, Russell, & Wilson, 2014). A critical step towards understanding how the current environment acts on individuals to shape patterns of parental care is to characterize the individual variation in responses to the environment.

First, there can be variation within individuals over time, associated with variation in their condition or context (i.e. plasticity). Plasticity refers to the capacity of individuals (or genotypes) to express different phenotypes in different environments (West-Eberhard, 2003). Selection may favour plasticity in parental care if plastic individuals can adaptively modify their behaviours to cope with a changing environment (Royle et al., 2014). Many studies have demonstrated average population plasticity in parental care in response to various environmental influences, including brood size and age (e.g. Westneat, Hatch, Wetzel, & Ensminger, 2011; Westneat, Mutzel, Bonner, & Wright, 2017), oxygen levels (e.g. Green & McCormick, 2005; Lissaker & Kvarnemo, 2006), sex change (Green & McCormick, 2005) and food resources (e.g. Krause, Krüger, & Pogány, 2017; Kvarnemo, Svensson, & Forsgren, 1998).

Second, there can be variation among individuals within a population (i.e. personality). Personality refers to consistent individual differences that are maintained across time and/or environmental contexts (Reale et al., 2007). Thus, plasticity and personality can refer to variation at different levels: within- and among-individuals (Westneat et al., 2011). Several explanations have been proposed to explain how selection can act to maintain among-individual variation (reviewed in Wolf & Weissing, 2010). For example, variation in past experiences can affect the optimal behavioural strategy in the current environment, which results in between-individual differences in behaviour (i.e. consistent individual differences can be a product of adaptive developmental

---

* Correspondence: T. A. Barbasch, Department of Biology and Marine Program, Boston University, 5 Cummington Mall, Boston, MA 02215, U.S.A.
E-mail address: tbarbasc@bu.edu (T. A. Barbasch).

https://doi.org/10.1016/j.anbehav.2017.12.002

plasticity; Stamps & Groothuis, 2010). Personality is a hot topic (Beekman & Jordan, 2017; Dall, Houston, & McNamara, 2004; Dingemanse & Wolf, 2010; Sih et al., 2015; Wolf & Weissing, 2012), but personality in parental care has rarely been explored. Several studies have, however, demonstrated repeatable among-individual differences in parental care across social and environmental contexts (e.g. Nakagawa, Gillespie, Hatchwell, & Burke, 2007; Schwagmeyer & Mock, 2003; Sprenger, Dingemanse, Dochtermann, Theobald, & Walker, 2012; Vallon et al., 2016).

A third possibility is that within- and among-individual variation exist simultaneously, such that individuals show repeatability in their behaviour through time but are flexible in their behaviour across contexts. We can integrate the study of plasticity and personality in parental care using the reaction norm approach (Dingemanse & Dochtermann, 2013; Dingemanse, Kazem, Réale, & Wright, 2010), which is equivalent to the character state approach in discrete environments (Via et al., 1995). Reaction norms are functions that describe how the phenotype of different genotypes changes across an environmental gradient, with each individual's phenotypic response fitted with an intercept and slope (Dingemanse & Wolf, 2013). Reaction norms can characterize the population mean response and the response of each individual through time and across environmental contexts. The study of plasticity and personality can thus be unified by quantifying variation in the slope and intercept of the population mean and individual reaction norms (Nussey, Wilson, & Brommer, 2007).

The reaction norm approach has rarely been used to study plasticity and personality in the context of parental care (Betini & Norris, 2012; Westneat et al., 2011), despite its utility in understanding how plasticity and personality explain patterns of variation in other contexts (Dingemanse et al., 2010). Here we apply the reaction norm framework to study variation in parental care in the clown anemonefish *Amphiprion percula*, within and among individuals. *Amphiprion percula* live in social groups with a larger, dominant female, a smaller, subordinate male, and up to four nonbreeding individuals (Buston & Wong, 2014). *Amphiprion* are protandrous hermaphrodites (Fricke & Fricke, 1977; Moyer & Nakazono, 1978): if the female of the group dies, then the male changes sex and assumes the position vacated by the female (Buston, 2004; Fricke, 1979; Mitchell, 2005). The breeding pair will lay a clutch of eggs up to three times per lunar month (Buston & Elith, 2011; Buston, 2004). Parents care in the form of tending, mouthing the eggs to remove debris and dead eggs, and fanning the eggs with their fins to oxygenate the clutch. In the congener *Amphiprion melanopus*, these parental behaviours are plastic and vary in response to day of development and sexual tactic of the individual (Green & McCormick, 2005). It is unknown whether there is among-individual variation in these behaviours also, but *A. percula* and its congener *Amphiprion ocellaris* exhibit consistent individual differences in activity, boldness and sociability (Medina & Buston, 2013; Schmiege, D'Aloia, & Buston, 2017; Wong et al., 2013). Our approach to studying within- and among-individual variation in *A. percula* will reveal whether and how parents vary in their responses to the environment.

Our objective was to characterize within- and among-individual variation in how *A. percula* parents respond to different resource environments. To accomplish this objective, we manipulated food resources available to pairs of *A. percula* and tested three hypotheses regarding variation in parental traits. First, we tested the hypothesis that there is plasticity in parental care (i.e. the average level of parental care varies across resource environments). If parents respond to resource availability, then we expected a main effect of environment on parental care. Second, we tested the hypothesis that there is personality in parental care (i.e. the individual level of parental care is repeatable over time). If parents exhibit consistently different behaviour through time, then we expected repeatable among-individual differences in intercept. Third, we tested the hypothesis that individuals vary in their response to the environment, and that the level of care and their responsiveness are related. If parents vary in their responsiveness, then we expected variation in the slope of individual reaction norms, and if the magnitude of an individual's response depends on its level of care, then we expected covariance between the slope and intercept. Taken together these tests provide a comprehensive characterization of within- and among-individual variation in parental care and provide insight into the proximate and ultimate causes of individual variation.

METHODS

Laboratory Population

We conducted this study at Boston University (Boston, MA, U.S.A.) from 27 June 2014 through 22 June 2015. All experimental fish originated from natural populations of *A. percula* in Papua New Guinea and were supplied by Quality Marine. When they arrived in the laboratory, all individuals were under 30 mm standard length (SL), ensuring that they were collected as nonbreeders in the wild. Removal of nonbreeders is considered to be a sustainable practice, because they are rapidly replaced and have no impact on population growth (e.g. Buston, 2004; Planes, Jones, & Thorrold, 2009; Schlatter, Webb, & Buston, n.d.). Upon arrival in our laboratory, we randomly paired each fish with one other fish and allowed them to establish dominance on their own (Wong, Uppaluri, Medina, Seymour, & Buston, 2016). At the start of this investigation, the individual fish had been in captivity for 3–4 years.

We maintained the laboratory population of 60 pairs (120 individuals) of *A. percula* in a large, recirculating aquarium system at Boston University in accordance with Institutional Animal Care and Use protocol (IACUC number 14-006). Fish were housed in pairs in 120-litre tanks with the set of 60 tanks divided into four independent racks with 15 tanks per rack. Each of the four racks had its own pump that supplied a continuous flow of salt water recirculating at a rate of approximately 16 600 litres/h. We used a Profilux computer controller to continually monitor the pH, temperature and salinity of water in each rack. We also manually tested water samples every 2 weeks for dissolved phosphate and ammonia (Salifert test kits, Amsterdam, The Netherlands; Red Sea test kits, www.redseafish.com). We maintained abiotic conditions as constant as possible, at levels similar to those found on coral reefs in Papua New Guinea: pH = 8.30 ± 0.34, temperature = 27.3 ± 0.19 °C, salinity = 32.5 ± 1.58 ppt. Each tank was lit with two T5 24 W bulbs whose spectra colour mimics the natural reef environment. Each tank contained 12 mm of sand on the bottom, a 15 × 15 cm ceramic tile, an anemone (*Entacmaea quadricolor*) and a small (approximately 10 × 10 cm) rock to provide habitat and substrate for egg laying. Prior to manipulation, we fed the fish approximately 24 commercial fish pellets (New Life Spectrum, New Life International, Inc., Homestead, FL, U.S.A.) per pair per day.

Feeding Manipulation

To determine how parental care varies across different environments, we conducted a feeding manipulation. Because pairs breed on a lunar schedule in the wild (Buston & Elith, 2011; Seymour, Barbasch, & Buston, 2017), the feeding manipulation ran for 12 lunar months from 27 June 2014 through 22 June 2015. Pairs were fed a high ration of food (24 fish pellets per pair per day) for 6 lunar months and a low ration of food (12 fish pellets per pair per day) for another 6 lunar months. Ten pairs received the high ration treatment for the first 6 months and the low ration for the following 6 months. The other 10 pairs received the low ration first, followed by the high ration. This allowed us to control for effects of treatment order on behaviour.

Reproduction

Of the 60 total pairs, 25 (42%) had laid a clutch of eggs at least once by the start of the manipulation. Of these 25, we chose 20 pairs that had bred together more than 10 times for the experimental manipulation. This allowed us to minimize the potential effects of early breeding experience, which positively influences reproductive output in the field (Buston & Elith, 2011) and in the laboratory (Barbasch & Buston, 2013). During the 12-month experiment, the 20 pairs laid between zero and three clutches per month, with an average of 1.4 clutches per pair per month. We observed a total of 155 clutches across the 20 pairs, 51 clutches in the low-ration treatment and 104 clutches in the high-ration treatment and measured parental care for all 155 clutches.

Parental Care

To investigate individual variation in parental behaviours, we quantified three metrics of care following Green and McCormick (2005): time tending, mouthing and fanning. Time tending is the amount of time a parent is within one body length of any part of the clutch; mouthing occurs when a parent places their mouth around one or several eggs; and fanning occurs when a parent waves its pectoral fins in the direction of the clutch.

For every clutch that was laid, we took 20 min videos of parental behaviour using high-resolution video cameras (GoPro Hero 4, GoPro Inc., San Mateo, CA, U.S.A.). The first 5 min of video served as an acclimation period and was discarded from our analysis, resulting in 15 min videos for data collection. Videos were taken at 14:00 hours on days 1, 4 and 7 of egg development. A preliminary study revealed no difference between levels of parental care in the morning and afternoon (Barbasch & Buston, 2013). Cameras were set up on a tripod outside the tank and facing the clutch. Videos were scored using JWatcher version 0.9 (http://www.jwatcher.ucla.edu). Based on our observations of behaviour, mouthing and fanning were discrete events lasting less than 3 s, therefore we compiled data as the amount of time out of the 15 min that the focal individual was tending, and the number of mouthing and fanning events per 15 min video. Parental behaviours were recorded separately for the male and female of each pair.

Statistical Analysis

Statistical analyses were performed in R version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). We analysed individual variation in parental care reaction norms using a mixed effects modelling approach (Dingemanse & Dochtermann, 2013; Dingemanse et al., 2010). Considering the dependent variables, parental behaviours (time tending, mouthing, fanning) were fitted in linear models using the ‘lme4’ package and degrees of freedom and *P* values were calculated using a Satterthwaite approximation using the ‘lmerTest’ package. For the continuous response variable time tending, we fitted a linear model with a Gaussian error distribution, and for the count response variables mouthing and fanning, we fitted linear models with Poisson error distributions and controlled for overdispersion by fitting an observation level random effect (Harrison, 2014).

Turning to consider the independent variables (fixed effects), feeding status (high or low ration) was mean-centred, such that the variation in intercept was expressed halfway between the high- and low-ration treatments, and set as the independent variable of primary interest. Treatment order was included to control for some pairs receiving the high ration first and some receiving the low ration first. Clutch age (a categorical variable with three levels: days 1, 4 and 7 after egg laying) and sex (male or female) were included because parental care increases throughout egg development and differs between the sexes in *A. melanopus* (Green & McCormick, 2005). All two-way interactions between feeding status, clutch age and sex were included because we suspected that the effect of one factor might vary with another. We used a Tukey test for all post hoc comparisons.

Turning to consider random effects, we fitted individual identity (ID) as a random intercept and the interaction between individual ID and feeding status as a random slope. We included sex as a grouping variable for individual ID to determine the among-individual variances for males and females. To control for repeated measures within each clutch, we fitted clutch ID, a unique identifier for each clutch observed, as an additional random intercept. Thus, we examined among- and within-individual variation through time and across feeding treatments by examining the extent to which individuals varied in the intercept and slope of their reaction norms.

To examine variation in parental care at the individual level, we used likelihood ratio tests (LRTs: -2 times the difference in log likelihood distributed as a chi-square statistic) to determine whether individual ID, sex as a grouping factor within individual ID, the interaction between individual ID and feeding status, and covariance between individual ID and feeding status would significantly improve model fit. This would indicate whether variation in those terms, respectively, significantly contributed to overall model variance. To further quantify variation among individuals, we estimated unadjusted repeatability as the proportion of the total variance accounted for by differences among individuals using the ‘rptR’ package in R (Nakagawa & Schielzeth, 2010). For the continuous variable tending, we estimated repeatability from our final model fitted with a Gaussian error distribution; for the count variables mouthing and fanning, we estimated repeatability from models fitted with Poisson error distributions using an additive overdispersion correction. The mixed effects modelling approach thus allowed us to comprehensively characterize the effect of environmental resources on individual variation in parental care.

RESULTS

Plasticity in Parental Care According to Sex

Parental care differed depending on the sex of the parent. Males and females differed in both the type and average level of care provided to their eggs. Males cared in the form of tending, mouthing and fanning, while females performed tending and mouthing but were not observed fanning. There was a significant main effect of sex on both tending and mouthing (Table 1, Fig. 1a and b). Males tended for an average (± SD) of 10.1 ± 3.3 min, mouthed an average of 35.0 ± 16.4 times and fanned their eggs an average of 39.4 ± 24.6 times per 15 min observation period. Females tended an average of 2.7 ± 2.8 min and mouthed an average of 3.9 ± 4.8 times. Thus, males performed an order of magnitude more parental care than females.

Plasticity in Parental Care in Response to Clutch Age

Parental care also differed as the clutch developed. There was a significant main effect of clutch age on all three metrics of parental care. Parents spent more time tending to, mouthing and fanning the eggs as the clutch developed from day 1 to day 7 (Table 1, Fig. 1a

Table 1
Summary of results for the fixed effects of the mixed model analyses

Model	Fixed effects	Estimate[a]	SE	*df*[b]	Test statistic	*P*
Tending	Feeding status	1.06	0.63	55	14.20	**0.026**
	Clutch age (4–1)	0.31	0.23	723	1.34	0.18
	Clutch age (7–1)	3.19	0.23	723	13.71	**<0.0001**
	Sex (F–M)	−6.37	0.67	32	−9.45	**<0.0001**
	Feeding status∗clutch age (4–1)	−0.08	0.34	729	−0.24	0.81
	Feeding status∗clutch age (7–1)	−0.31	0.34	729	−0.91	0.36
	Feeding status∗sex (F–M)	0.70	0.55	29	1.27	0.21
	Clutch age (4–1)∗sex (F–M)	−0.32	0.32	717	−1.00	0.31
	Clutch age (7–1)∗sex (F–M)	−2.80	0.32	717	−8.79	**<0.0001**
	Treatment order	−0.49	0.57	31	−0.87	0.39
Mouthing	Feeding status	0.04	0.11	55	0.39	0.69
	Clutch age (4–1)	0.02	0.02	723	0.96	0.33
	Clutch age (7–1)	0.17	0.02	723	8.37	**<0.0001**
	Sex (F–M)	−2.15	0.14	32	−15.02	**<0.0001**
	Feeding status∗clutch age (4–1)	0.05	0.04	729	1.15	0.25
	Feeding status∗clutch age (7–1)	−0.22	0.04	729	−5.62	**<0.0001**
	Feeding status∗sex (F–M)	0.57	0.16	29	3.52	**0.0004**
	Clutch age (4–1)∗sex (F–M)	−0.52	0.06	717	−8.30	**<0.0001**
	Clutch age (7–1)∗sex (F–M)	−0.40	0.06	717	−6.84	**0.0001**
	Treatment order	0.03	0.11	31	0.24	0.81
Fanning (male only)	Feeding status	0.37	0.37	65	1.01	0.31
	Clutch age (4–1)	1.90	0.19	303	9.89	**<0.0001**
	Clutch age (7–1)	4.64	0.18	302	25.27	**<0.0001**
	Feeding status∗clutch age (4–1)	1.02	0.38	303	2.65	**0.008**
	Feeding status∗clutch age (7–1)	0.94	0.36	302	0.26	0.80
	Treatment order	−0.85	0.37	19	−2.26	**0.02**

Results are shown for three models with dependent variables tending, mouthing and fanning. Models include the independent variables feeding status (high ration: H; low ration: L), clutch age (days 1, 4, 7), sex (female: F; male: M), the interaction between feeding status and clutch age, the interaction between feeding status and sex, and the interaction between sex and clutch age. (Sex and its interactions are not included for fanning, because only males fanned). Individual identity (ID), the interaction between individual ID and feeding status and clutch ID were fitted as random effects. Shown are the fixed effects, coefficient estimates, test statistic values and *P* values.

[a] Estimates for models of mouthing and fanning are shown on the log link scale.

[b] Degrees of freedom were calculated using a Satterthwaite approximation.

and b). Thus, for all three metrics of care, there was an increase in average level of care at later stages of development.

Plasticity in Parental Care in Response to Resource Environment

There was a significant main effect of feeding treatment on tending, and a significant interaction effect between feeding treatment and sex (Table 1, Fig. 1a). The main effect indicates that individuals spent on average 0.97 more minutes tending when they were fed the high ration compared to the low ration. Post hoc comparisons revealed that males did not exhibit plasticity in response to feeding treatment (Tukey: estimate = 1.06, $z = 2.24$, $P = 0.10$) but females spent on average 1.78 more minutes tending, or 87% more time tending, when fed a high compared to a low ration (Tukey: estimate = 1.78, $z = 3.81$, $P = 0.001$).

There was no significant main effect of feeding treatment on mouthing, but there was a significant interaction between feeding treatment and sex (Table 1, Fig. 1b). The interaction effect indicates that the effect of feeding treatment varied by sex. Specifically, post hoc comparisons revealed that males did not exhibit plasticity in mouthing across feeding treatment (Tukey: log scale estimate = 0.03, $z = -0.25$, $P = 0.99$), but females mouthed on average 1.81 more times, or 53% more, when fed a high compared to the low ration (Tukey: log scale estimate = 0.60, $z = 4.15$, $P = 0.0002$).

There was no significant main effect of feeding treatment on fanning for males, but there was a significant interaction between feeding treatment and clutch age (Table 1, Fig. 1c; females did not fan). The interaction effect revealed that the effect of feeding treatment varied by clutch age for males. Specifically, post hoc comparisons revealed that males did not exhibit plasticity in response to higher food ration on day 1 (Tukey: log scale estimate = 0.16, $z = 0.36$, $P = 0.99$) or day 7 (Tukey: log scale estimate = 0.55, $z = 2.05$, $P = 0.30$) of development, but fanned on average four more times, or 330% more, in response to higher food ration on day 4 of development (Tukey: log scale estimate = 1.40, $z = -4.29$, $P < 0.001$). We found a similar pattern when comparing day 7 versus day 1 and day 4 versus day 1 (Fig. 1c), but due to the large amount of variation in fanning on day 7, the effect was not significant (Table 1).

Personality in Parental Care

We measured the among-individual variation in parental care by fitting individual ID as a random intercept and including sex as a grouping variable for the behaviours exhibited by both sexes (tending and mouthing) (Fig. 2). Including individual ID as a random intercept in our models resulted in a significantly better fit for all parental care metrics (Table 2), indicating significant repeatability in all three metrics of care. Because we mean-centred our environmental variable (feeding status), the significant variation in intercept was expressed midway between high and low ration and indicated repeatable variation in parental behaviour through time. Repeatabilities were calculated as the proportion of total variation accounted for by between-individual differences, thus a repeatability score close to 1 indicates that most of the variation in the response variable is due to among-individual variation as opposed to within-individual variation. Repeatability was 0.73 for tending, 0.84 for mouthing and 0.08 for fanning. Including among-individual variance by sex significantly improved model fit for mouthing (LRT: $\chi^2 = 9.04$, $P = 0.03$) but not tending (LRT: $\chi^2 = 4.23$, $P = 0.24$). Among-individual variance in mouthing was 0.05 for males and 0.29 for females.

Plasticity and Personality in Parental Care

Figure 2 summarizes the interplay between plasticity and personality, showing the population mean response to resource

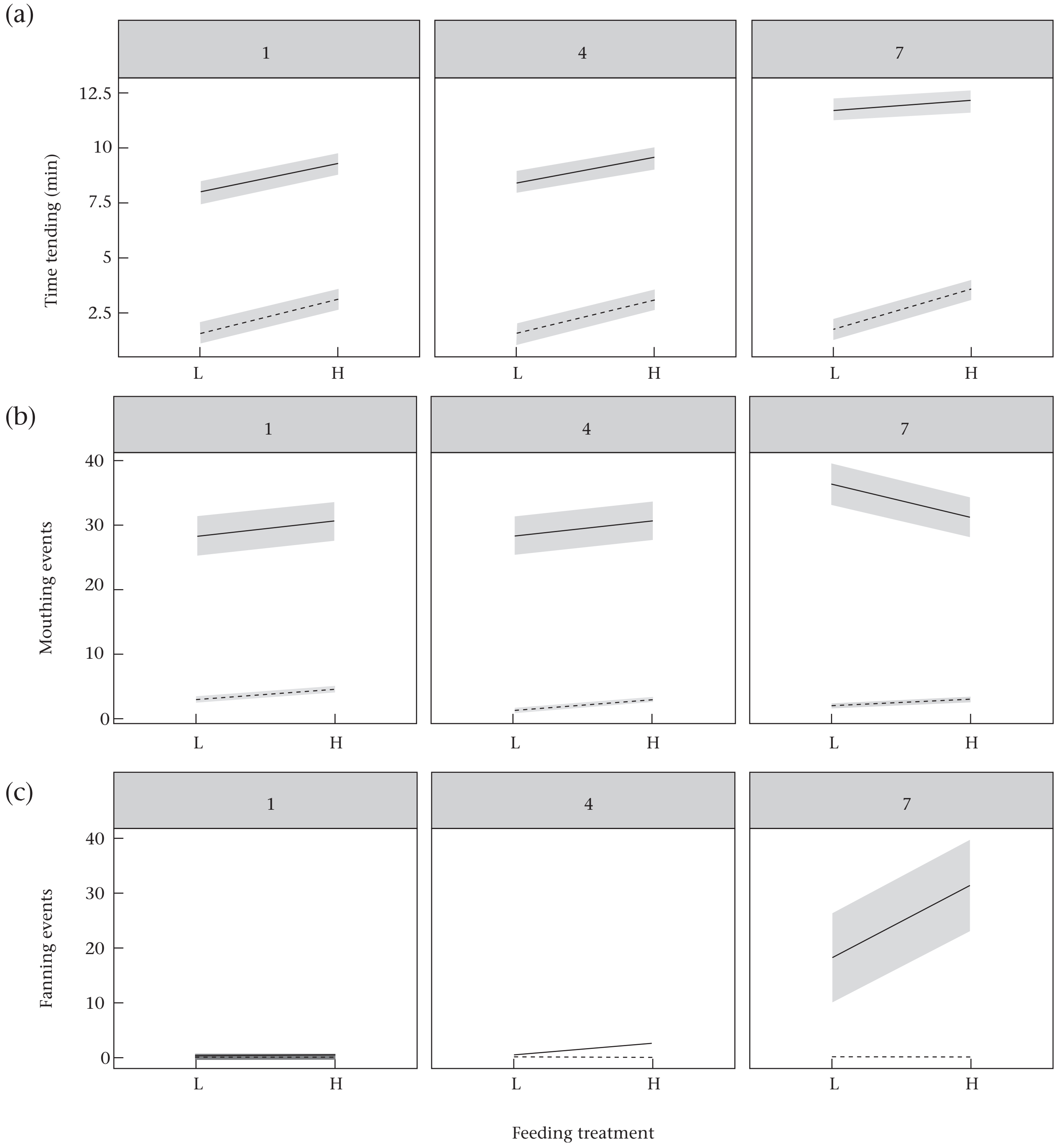

Figure 1. Average parental care reaction norms across feeding treatments (low ration: L; high ration: H) and clutch development (days 1, 4 and 7 of egg development). Shown are means and standard errors from models of (a) tending, (b) mouthing and (c) fanning. Solid lines represent reaction norms for males, dashed lines represent reaction norms for females, and shaded areas represent standard errors around each slope estimate. Reaction norms and standard errors were generated using least square means and standard errors from post hoc comparisons of the interactions between feeding treatment, sex and clutch age in our models.

availability along with individual reaction norms. Significant variation in slope of the reaction norms describes among-individual variation across feeding treatments. Fitting feeding treatment as a random slope significantly improved model fit for tending and mouthing but not for fanning, indicating among-individual variation in the plastic response across feeding treatment for tending and mouthing (Table 2). Including covariance between random slope and intercept in these models, however, did not significantly improve model fit for any of the parental care metrics (LRTs: $P > 0.05$). This indicates that while variation in the slope and intercept in parental care coexist, their covariance did not significantly contribute to between-individual differences in behaviour.

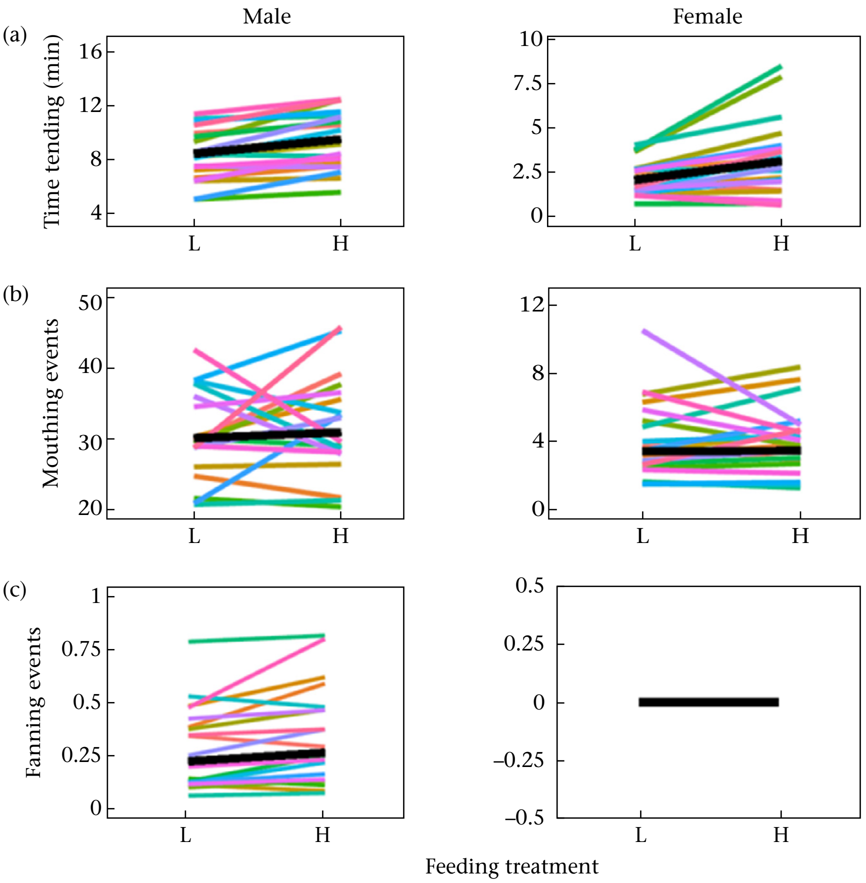

Figure 2. Individual parental care reaction norms across feeding treatments (low ration: L; high ration: H) for males and females. Shown are individual reaction norms from models for (a) tending, (b) mouthing and (c) fanning. Thin lines represent individual reaction norms fitted for each individual within a sex, with an intercept fitted from multiple measures through time and a slope fitted to measures across feeding environments for each individual. Thick lines represent the average population reaction norms across all individuals of that sex. Reaction norms were generated with the individual intercept and slope estimates from our models. Note that male and female behaviours are on different scales.

Table 2
Results for likelihood ratio tests of the random intercept, slope and intercept–slope covariance for each parental care model

Model	Random effect	χ^2	*df*	Random variance	Residual variance	Intercept–slope covariance	*P*
Tending	Intercept	292.1	1	3.55			**<0.0001**
	Slope	29.57	2	2.19	3.82	0.29	**<0.0001**
	Covariance	1.95	1				0.16
Mouthing	Intercept	151.7	1	0.17			**<0.0001**
	Slope	17.46	2	0.16	NA[a]	−0.27	**0.0002**
	Covariance	0.87	1				0.35
Fanning	Intercept	51.2	1	0.88			**<0.0001**
	Slope	1.87	2	0.35	NA[a]	0.16	0.39
	Covariance	0.13	1				0.72

Tests determine whether including individual identity (ID) as a random intercept, the interaction between individual ID and feeding status, and the covariance between the slope and intercept significantly improved the fit of the models of tending, mouthing and fanning. Shown are the chi-square statistic, degrees of freedom, variance estimate of the random intercept and slope, residual variance, intercept–slope covariance and *P* value.

[a] Residual variances not applicable; calculated only for Gaussian error distributions.

DISCUSSION

Parental care is highly variable within and among individuals, yet few studies have explored the role of individual variation in shaping patterns of care (Betini & Norris, 2012; Royle et al., 2014; Westneat et al., 2011). Here we applied a reaction norm approach to determine how parents vary in their responses to resource availability in *A. percula* and discuss the potential proximate and ultimate causes of this variation. We found significant variation among the sexes, within individuals through time and among individuals across contexts in response to the environment. Parents demonstrated variation within individuals (plasticity) in multiple aspects of parental care, and this differed depending on the sex of the individual: parents generally increased tending, fanning and mouthing with increased resource availability, but this response was significantly affected by clutch age and sex. Parents also demonstrated among-individual variance that was maintained through time (personality) in all behaviours: the behaviour of individuals was sufficiently repeatable to generate significant between-individual differences in the amount of care provided.

Repeatability was high for tending (0.73) and mouthing (0.83) but low for fanning (0.08). For mouthing, males and females differed not only in the amount of care provided, but also in among-individual variance in care, with males displaying lower among-individual variance than females. Finally, individuals were variable in the slope and elevation of their parental care reaction norms (plasticity and personality) for tending and mouthing but only variable in elevation for fanning, revealing that there was interindividual variation in plastic responses to resource availability. Each of these results has implications for the mechanisms driving within- and among-individual variation in parental care.

Plasticity in Parental Care

Parental care is given at the expense of other activities, such as foraging or investment in future reproduction. This trade-off can influence the costs and benefits of providing care and thus favour plasticity, such that individuals adaptively modify their behaviour in response to changing environmental conditions (Royle et al., 2014; West-Eberhard, 2003). Here we found that, for most metrics of care that we measured, *A. percula* increased parental behaviours with an increase in food resources. Females increased their time tending and mouthing when fed a high-ration compared to a low-ration of food. Males increased fanning with increased food resources, but not at all stages of egg development. Parental care tended to increase with an increase in resources (i.e. the plasticity was positive), building on the result that pairs of *A. percula* in high-resource environments seem to have higher reproductive success than pairs in low-resource environments in the field (Buston & Elith, 2011).

In addition to showing a plastic response to resource availability, individuals also showed a plastic response to the age of the clutch and their sex. Average levels of tending, mouthing and fanning were greater at later stages of clutch development (Fig. 2; consistent with Green & McCormick, 2005). This increase in parental behaviours as the clutch neared hatching may be a result of parents responding to the increased metabolic needs of the offspring later in development (Green & McCormick, 2005; Green, 2004). Average levels of tending, mouthing and fanning were higher for males than for females (Fig. 1; consistent with Green & McCormick, 2005). Because *Amphiprion* spp. change sex from male to female (Fricke & Fricke, 1977; Moyer & Nakazono, 1978), this is indicative of plasticity as a function of sex. Interestingly, in addition to sex differences in mean behaviour, there was also a difference in among-individual variance of male and female behaviour. Females displayed significantly greater among-individual variance in mouthing behaviour than males. These changes in parental behaviours with sex may be a result of the sexes being under different selection and/or a parental division of labour.

Personality in Parental Care

While plasticity in parental care provides the opportunity for individuals to adaptively adjust their behaviour to a changing environment, it is less intuitive why consistent individual differences would be maintained through time and context (Bell, 2007). Explanations for the adaptive value of among-individual variation generally rely on the existence of stable differences in state. Differences in parental state (such as age, health or size) could influence the costs and benefits of parenting behaviours, which in turn could favour different parenting strategies among different individuals (Bell, 2007; Dingemanse & Wolf, 2010; Stamps, 2007). In *A. percula*, there is evidence that the early environment acts as a ‘silver spoon’ such that individuals that are lucky enough to be born/recruited into good environments gain multiple benefits (Buston & Elith, 2011; Salles et al., 2016), which could provide a mechanism that generates state differences in this species.

Alternatively, in sex-changing species, repeatable differences in behaviour among individuals could be generated by intralocus sexual conflict if the optimal level of behaviour differs for males and females (Sprenger et al., 2012). *Amphiprion percula* change sex from male to female, and the sexes differ in both their average parental care and among-individual variance in care. Assuming parental behaviour has a genetic component, individuals may be unable to exhibit the optimal behaviour in both sexes, resulting in individuals adopting suboptimal but equally fit behavioural strategies. The extent to which genetics and developmental plasticity contribute to this variation in parental care remains an unanswered question.

In addition to the role of genetics and development, the social environment may contribute to personality variation under certain conditions. For example, a model developed by McNamara, Stephens, Dall, and Houston (2009) revealed how feedback between a trait and the monitoring of that trait by a social partner can maintain variation among individuals. If some individuals are more cooperative than others, that should favour the monitoring of a social partner's behaviour. If monitoring is costly, however, this can select for variation in whether or not individuals monitor their partners, which in turn can select for variation in their partner's level of cooperation (McNamara et al., 2009). In the context of parental care, parents care together for each clutch and therefore individuals may be able to monitor the care of their partner with a cost in terms of time and energy spent monitoring, which in turn could select for variation in their partner's behaviour. While multiple adaptive explanations for personality have been proposed (Dingemanse & Wolf, 2010), specific tests of the assumptions and predictions of these models are needed to understand how and why consistent individual differences exist in parental care.

Plasticity and Personality in Parental Care

We have demonstrated that there can be both average population-level plasticity and repeatable individual differences in parental behaviour through time. Within the reaction norm framework, this effect is shown as a nonzero slope in the average reaction norm and differences in the elevation of individual reaction norms. These measures of plasticity and personality, however, do not account for the total variation because reaction norm slope and elevation can vary simultaneously. We captured this by characterizing the individual variation in slope across feeding treatments and covariance between slope and intercept. In biological terms, significant individual variation in slope suggests that there is among-individual variation in the behavioural response to feeding treatment and a significant covariance between slope and elevation suggests that the amount of plasticity in a trait depends on the trait value.

Parents demonstrated significant between-individual differences in the slopes of the reaction norms for time tending. This suggests that not all individuals respond to changes in the environment in the same way. Similarly, individuals showed consistent between-individual differences in the slopes for mouthing. Considering proximate causes, individual variation in slope can result if past environmental conditions or genetic differences influence how parents respond to changes in resource availability. Indeed, an evolutionary model developed by Wolf, van Doorn, and Weissing (2008) suggests that if the benefits of plasticity are negatively frequency dependent, responsive and unresponsive individuals can be maintained in a population. Here we have shown that even in circumstances with no average population plasticity, there can still be substantial variation at the individual level. Studying this unexplored level of variation as well as the heritability of parental traits is critical to understanding the evolution and maintenance of observed patterns of parental care (Nussey et al., 2007; Royle et al., 2014).

We have shown that the slope and intercept of parental care reaction norms can vary simultaneously, but we found no evidence that slope and intercept covary. Thus, the amount of among-individual variation, measured as repeatability, did not change depending on whether individuals were fed a high or a low ration. A similar pattern of high variation in the slope and intercept, but low covariance between them, has been demonstrated in several other species (reviewed in Nussey et al., 2007). Correlations between the level of a trait and plasticity can potentially impose a constraint on the evolution of the trait if there is an underlying genetic correlation (Dingemanse & Wolf, 2013). In *A. percula*, there is substantial among- and within- individual variation in parental care, but we did not detect any relationship between the amount of parental care exhibited by an individual and its responsiveness to the environment; thus, selection may be acting separately on these two phenotypic components.

Conclusions

In this study we applied a reaction norm framework to describe how individual parents vary in their response to environmental resource availability in *A. percula*. Understanding this variation is essential to studies of behaviour, as there is potentially substantial variation not accounted for when considering only the population mean and variance values. This variation in turn will have consequences for how we think about adaptive plasticity and its role in the evolution of parental care. Here we present a framework for systematically studying how individual parents respond to their environment. By characterizing variation in parental care at the individual level, this framework will lay the foundation for studies of the mechanisms giving rise to and maintaining this variation in clownfish as well as other systems. Furthermore, studying the heritability of such variation will provide insight into the evolution of plasticity and personality. Future applications of this framework thus have the potential to expose unexplored phenotypic variation and provide a foundation for studying how selection shapes and maintains patterns of parental care within and across species.

Acknowledgments

We thank J. Seymour, L. Wong, and J. Moore for help in the lab and K. Warkentin, S. Alonzo, M. Sorenson, and G. Jones for helpful comments and discussion. We also thank D. Westneat and K. Heubel for their insightful reviews. This research was made possible by an NSF Graduate Research Fellowship Award and a Warren McLeod grant to T. Barbasch and a start-up grant awarded by the Trustees of Boston University to P. Buston.

References

Barbasch, T. A., & Buston, P. M. (2013). *Determinants of reproductive success of the laboratory population used in the present study*. Unpublished raw data.

Beekman, M., & Jordan, L. A. (2017). Does the field of animal personality provide any new insights for behavioral ecology? *Behavioral Ecology, 28*(3), 617–623.

Bell, A. M. (2007). Evolutionary biology: Animal personalities. *Nature, 447*(7144), 539–540. http://doi.org/447539a [pii] 10.1038/447539a.

Betini, G. S., & Norris, D. R. (2012). The relationship between personality and plasticity in tree swallow aggression and the consequences for reproductive success. *Animal Behaviour, 83*, 137–143. https://doi.org/10.1016/j.anbehav.2011.10.018.

Buston, P. (2004). Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish *Amphiprion percula*. *Behavioral Ecology and Sociobiology, 57*(1), 23–31. https://doi.org/10.1007/s00265-004-0833-2.

Buston, P. M., & Elith, J. (2011). Determinants of reproductive success in dominant pairs of clownfish: A boosted regression tree analysis. *Journal of Animal Ecology, 80*(3), 528–538. https://doi.org/10.1111/j.1365-2656.2011.01803.x.

Buston, P. M., & Wong, M. Y. L. (2014). Why some animals forgo reproduction in complex societies. *American Scientist, 102*(4), 290–297. https://doi.org/10.1511/2014.109.290.

Dall, S. R. X., Houston, A. I., & McNamara, J. M. (2004). The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecology Letters, 7*(8), 734–739. https://doi.org/10.1111/j.1461-0248.2004.00618.x.

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *Journal of Animal Ecology, 82*(1), 39–54. https://doi.org/10.1111/1365-2656.12013.

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution, 25*(2), 81–89. https://doi.org/10.1016/j.tree.2009.07.013.

Dingemanse, N. J., & Wolf, M. (2010). Recent models for adaptive personality differences: A review. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1560), 3947–3958. https://doi.org/10.1098/rstb.2010.0221.

Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: Causes and consequences. *Animal Behaviour, 85*, 1031–1039. https://doi.org/10.1016/j.anbehav.2012.12.032.

Fricke, H. (1979). Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos*. *Ethology, 50*(3), 313–326.

Fricke, H., & Fricke, S. (1977). Monogamy and sex change by aggressive dominance in coral reef fish. *Nature, 266*, 830–832.

Green, B. S. (2004). Embryogenesis and oxygen consumption in benthic egg clutches of a tropical clownfish, *Amphiprion melanopus* (Pomacentridae). *Comparative Biochemistry and Physiology A, Molecular and Integrative Physiology, 138*(1), 33–38. https://doi.org/10.1016/j.cbpb.2004.02.014.

Green, B. S., & McCormick, M. I. (2005). O2 replenishment to fish nests: Males adjust brood care to ambient conditions and brood development. *Behavioral Ecology, 16*(2), 389–397. https://doi.org/10.1093/beheco/ari007.

Harrison, X. A. (2017). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *Peer J, 2*, e616.

Krause, E. T., Krüger, O., & Pogány, Á. (2017). Zebra finch nestlings, rather than parents, suffer from raising broods under low nutritional conditions. *Behavioral Ecology and Sociobiology, 71*(10), 152.

Kvarnemo, C., Svensson, O. L. A., & Forsgren, E. (1998). Parental behaviour in relation to food availability in the common goby. *Animal Behaviour, 56*, 1285–1290.

Lissåker, M., & Kvarnemo, C. (2006). Ventilation or nest defense—parental care trade-offs in a fish with male care. *Behavioral Ecology and Sociobiology, 60*(6), 864–873.

McNamara, J. M., Stephens, P. A., Dall, S. R., & Houston, A. I. (2009). Evolution of trust and trustworthiness: Social awareness favours personality differences. *Proceedings of the Royal Society B: Biological Sciences, 276*(1657), 605–613. https://doi.org/10.1098/rspb.2008.1182.

Medina, A., & Buston, P. M. (2013). *Plasticity in personality traits in response to changes in social context in the clown anemonefish*. Unpublished raw data.

Mitchell, J. (2005). Queue selection and switching by false clown anemonefish, *Amphiprion ocellaris*. *Animal Behaviour, 69*, 643–652. https://doi.org/10.1016/j.anbehav.2004.05.017.

Moyer, J. T., & Nakazono, A. (1978). Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. *Japanese Journal of Ichthyology, 25*(2), 101–106.

Nakagawa, S., Gillespie, D. O. S., Hatchwell, B. J., & Burke, T. (2007). Predictable males and unpredictable females: Sex difference in repeatability of parental care in a wild bird population. *Journal of Evolutionary Biology, 20*(5), 1674–1681. https://doi.org/10.1111/j.1420-9101.2007.01403.x.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: A practical guide for biologists. *Biological Reviews, 85*(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x.

Nussey, D. H., Wilson, A. J., & Brommer, J. E. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology, 20*(3), 831–844. https://doi.org/10.1111/j.1420-9101.2007.01300.x.

Planes, S., Jones, G. P., & Thorrold, S. R. (2009). Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences of the United States of America, 106*(14), 5693–5697. https://doi.org/10.1073/pnas.0808007106.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x.

Royle, N. J., Russell, A. F., & Wilson, A. J. (2014). The evolution of flexible parenting. *Science, 345*(6198), 776–781. https://doi.org/10.1126/science.1253294.

Royle, N. J., Smiseth, P. T., & Kölliker, M. (2012). *The evolution of parental care*. Oxford, U.K.: Oxford University Press.

Salles, O. C., Saenz-Agudelo, P., Almany, G. R., Berumen, M. L., Thorrold, S. R., Jones, G. P., et al. (2016). Genetic tools link long-term demographic and life-history traits of anemonefish to their anemone hosts. *Coral Reefs, 35*(4), 1127–1138. https://doi.org/10.1007/s00338-016-1485-1.

Schlatter, E., Webb, C. T. W., & Buston, P. M.. (n.d.). Effect of alternative harvesting strategies on the population growth of the clown anemonefish *Amphiprion percula*. Manuscript in preparation.

Schmiege, P. F., D'Aloia, C. C., & Buston, P. M. (2017). Anemonefish personalities influence the strength of mutualistic interactions with host sea anemones. *Marine Biology, 164*(1), 24.

Schwagmeyer, P. L., & Mock, D. W. (2003). How consistently are good parents good parents? Repeatability of parental care in the house sparrow, *Passer domesticus*. *Ethology, 109*(4), 303–313. https://doi.org/10.1046/j.1439-0310.2003.00868.x.

Seymour, J. R., Barbasch, T. A., & Buston, P. M. (2017). *Lunar cycles of reproduction in the clown anemonefish* Amphiprion percula*: Individual-level strategies and population-level patterns*. Manuscript submitted for publication.

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P. O., Wolf, M., & Dingemanse, N. J. (2015). Animal personality and state–behaviour feedbacks: A review and guide for empiricists. *Trends in Ecology & Evolution, 30*(1), 50–60. https://doi.org/10.1016/j.tree.2014.11.004.

Sprenger, D., Dingemanse, N. J., Dochtermann, N. A., Theobald, J., & Walker, S. P. W. (2012). Aggressive females become aggressive males in a sex-changing reef fish. *Ecology Letters, 15*(9), 986–992. https://doi.org/10.1111/j.1461-0248.2012.01819.x.

Stamps, J. A. (2007). Growth-mortality tradeoffs and personality traits in animals. *Ecology Letters, 10*(5), 355–363. https://doi.org/10.1111/j.1461-0248.2007.01034.x.

Stamps, J. A., & Groothuis, T. G. G. (2010). The development of animal personality: Relevance, concepts and perspectives. *Biological Reviews, 85*(2), 301–325. https://doi.org/10.1111/j.1469-185X.2009.00103.x.

Trivers, R. L. L. (1972). Parental investment and sexual selection. In *Sexual selection and the descent of man* (pp. 53–95). Chicago, IL: Aldine.

Vallon, M., Grom, C., Kalb, N., Sprenger, D., Anthes, N., Lindström, K., et al. (2016). You eat what you are: Personality-dependent filial cannibalism in a fish with paternal care. *Ecology and Evolution, 6*(5), 1340–1352. https://doi.org/10.1002/ece3.1966.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., & Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: Consensus and controversy. *Trends in Ecology & Evolution, 10*(5), 212–217.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford, U.K.: Oxford University Press.

Westneat, D. F., Hatch, M. I., Wetzel, D. P., & Ensminger, A. L. (2011). Individual variation in parental care reaction norms: Integration of personality and plasticity. *American Naturalist, 178*(5), 652–667. https://doi.org/10.1086/662173.

Westneat, D. F., Mutzel, A., Bonner, S., & Wright, J. (2017). Experimental manipulation of brood size affects several levels of phenotypic variance in offspring and parent pied flycatchers. *Behavioral Ecology and Sociobiology, 71*, 91. https://doi.org/10.1007/s00265-017-2315-3.

Wolf, M., van Doorn, G. S., & Weissing, F. J. (2008). Evolutionary emergence of responsive and unresponsive personalities. *Proceedings of the National Academy of Sciences of the United States of America, 105*(41), 15825–15830. https://doi.org/10.1073/pnas.0805473105.

Wolf, M., & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1560), 3959–3968. https://doi.org/10.1098/rstb.2010.0215.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology & Evolution, 27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001.

Wong, M. Y. L., Medina, A., Uppaluri, C., Arnold, S., Seymour, J. R., & Buston, P. M. (2013). Consistent behavioural traits and behavioural syndromes in pairs of the false clown anemonefish *Amphiprion ocellaris*. *Journal of Fish Biology, 83*(1), 207–213. https://doi.org/10.1111/jfb.12133.

Wong, M. Y. L., Uppaluri, C., Medina, A., Seymour, J., & Buston, P. M. (2016). The four elements of within-group conflict in animal societies: An experimental test using the clown anemonefish, *Amphiprion percula*. *Behavioral Ecology and Sociobiology, 70*(9), 1467–1475. https://doi.org/10.1007/s00265-016-2155-6.