

Determinants of reproductive success in dominant pairs of clownfish: a boosted regression tree analysis

Peter M. Buston^{1*} and Jane Elith²

¹Department of Biology and Marine Program, Boston University, 5 Cummington Avenue, Boston, MA 02215, USA; and

²School of Botany, University of Melbourne, Parkville, Vic. 3010, Australia

Summary

1. Central questions of behavioural and evolutionary ecology are what factors influence the reproductive success of dominant breeders and subordinate nonbreeders within animal societies? A complete understanding of any society requires that these questions be answered for all individuals.

2. The clown anemonefish, *Amphiprion percula*, forms simple societies that live in close association with sea anemones, *Heteractis magnifica*. Here, we use data from a well-studied population of *A. percula* to determine the major predictors of reproductive success of dominant pairs in this species.

3. We analyse the effect of multiple predictors on four components of reproductive success, using a relatively new technique from the field of statistical learning: boosted regression trees (BRTs). BRTs have the potential to model complex relationships in ways that give powerful insight.

4. We show that the reproductive success of dominant pairs is unrelated to the presence, number or phenotype of nonbreeders. This is consistent with the observation that nonbreeders do not help or hinder breeders in any way, confirming and extending the results of a previous study.

5. Primarily, reproductive success is negatively related to male growth and positively related to breeding experience. It is likely that these effects are interrelated because males that grow a lot have little breeding experience. These effects are indicative of a trade-off between male growth and parental investment.

6. Secondly, reproductive success is positively related to female growth and size. In this population, female size is positively related to group size and anemone size, also. These positive correlations among traits likely are caused by variation in site quality and are suggestive of a silver-spoon effect.

7. Noteworthy, whereas reproductive success is positively related to female size, it is unrelated to male size. This observation provides support for the size advantage hypothesis for sex change: both individuals maximize their reproductive success when the larger individual adopts the female tactic.

8. This study provides the most complete picture to date of the factors that predict the reproductive success of dominant pairs of clown anemonefish and illustrates the utility of BRTs for analysis of complex behavioural and evolutionary ecology data.

Key-words: cooperative breeding, eusociality, group augmentation, life-history trade-off, machine learning, maternal effects, parental investment, sex change, silver spoon, size advantage

Introduction

Animal societies in which dominant individuals monopolize reproduction while subordinates delay or forego reproduction are one of the most remarkable products of evolution – remarkable, because it is not immediately

apparent why these societies are stable, given the reproductive conflicts that are inherent within them (Emlen 1997). A complete understanding of any society requires that the fitness costs and benefits of the association are understood from the perspective of each individual. It is notoriously difficult to get at the factors that influence the reproductive success of dominant individuals, because characteristics of dominants, their subordinates and their territories are

*Correspondence author. E-mail: buston@bu.edu

often positively correlated (Brown *et al.* 1982; Cockburn 1998; Dickinson & Hatchwell 2004; Wright & Russell 2008). There are two common solutions to this problem: (i) to use multivariate analyses to investigate the effect of one factor while statistically controlling for the effect of other factors; and (ii) to use experimental manipulations to determine the effect of manipulating one factor while leaving other factors unchanged (Emlen & Wrege 1991; Mumme 1992; Komdeur 1994; Buston 2004a; Brouwer, Heg & Taborsky 2005; Cockburn *et al.* 2008). The trouble is experimental manipulations rarely leave other factors unchanged, and statistical analyses are often limited by their assumptions regarding underlying data distributions (e.g. normality or homogeneity of variance) or investigator assumptions about the nature of relationships (e.g. linear or curvilinear). New statistical methods for analysing reproductive success that do not make, or force investigators to make, such assumptions could contribute much to the field.

The majority of work on animal societies has been conducted on terrestrial organisms, and testing the generality of the findings requires extending the taxonomic and environmental scope of research (Emlen 1991). The clown anemonefish, *Amphiprion percula*, is contributing to our understanding of the evolution of animal societies (Buston 2002). In Madang Lagoon, Papua New Guinea, clownfish live in close association with the anemone *Heteractis magnifica* (Fautin 1992; Elliott, Elliott & Mariscal 1995; Elliott & Mariscal 2001). Within each anemone, there is a single group of fish, composed of a dominant breeding pair and zero to four nonbreeders (Buston 2003a, 2004a). The groups are not composed of relatives, and nonbreeders do not help in any obvious way (Buston 2004a; Buston *et al.* 2007). Within each group, there is a size-based dominance hierarchy: the female is largest, the male is second largest, and nonbreeders get progressively smaller (Buston 2003b; Buston & Cant 2006). The size hierarchy represents a queue for breeding positions: if the female of the group dies, then the males change sex and assume the position vacated by the female, and the largest nonbreeder inherits the position vacated by the sex-changing male (Buston 2003b, 2004b; also Mitchell 2005). Reproduction occurs year round, and breeders hatch hundreds of eggs each lunar month, but the factors influencing reproductive success are only minimally understood (Buston 2004a; also Mitchell 2003). *A. percula* represent a case of the classic problem, in which characteristics of dominants, their subordinates and their territories are all positively correlated (Fautin 1992; Elliott & Mariscal 2001; Buston 2003a,b; see also Mitchell & Dill 2005).

Here, to gain a greater understanding the determinants of reproductive success in dominant pairs of *A. percula*, we use a relatively new technique from the field of statistical learning: Boosted Regression Trees (BRTs). The salient features of BRTs are that they can accommodate any type of variable (continuous, categorical, also missing and non-independent data) and can deal with highly correlated sets of independent variables; BRTs identify important predictor variables and

enable complex functions and their interactions to be modelled without making assumptions about the type of functions or interactions (Elith, Leathwick & Hastie 2008). We use BRTs to investigate the major predictors of four components of reproductive success of dominant pairs of *A. percula*: (i) the probability of laying eggs; (ii) the number of eggs laid; (iii) the probability of hatching eggs; and (iv) the proportion of eggs hatched. These analyses enable us to identify the individual, social, ecological, spatial and temporal factors that influence each component of reproductive success. Further, we investigate the major predictors of the total number of eggs hatched per lunar month. This final analysis enables us to see which of the factors identified in the four preceding analyses have the strongest effect on overall reproductive success. Also, this latter analysis permits direct comparison of the boosted regression tree analysis presented here with the mixed model analysis presented elsewhere (Buston 2004a). This study provides the most complete picture to date of the factors that determine anemonefish reproductive success and reproductive strategies and illustrates the utility of BRTs for analysis of complex behavioural and evolutionary ecology data.

Materials and methods

STUDY POPULATION

This investigation was conducted using data from a population of clown anemonefish *A. percula*, in Madang Lagoon, Papua New Guinea, which was studied from January 1997 to December 1997 (Buston 2002). All fieldwork was conducted using SCUBA, at depths of less than 15 m. Seventy-one anemones (*Heteractis magnifica*) were located on two reefs: Sinub (reef 1), $n = 40$; Wongad (reef 2), $n = 31$. [Anemones on a third reef (Masamoz, $n = 26$), used in Buston 2004a,b; were not included in this study, because reproduction was not monitored as rigorously on this third reef]. Each anemone was occupied by a single group of *A. percula*. Groups consisted of a dominant pair and zero to four subordinate nonbreeders. Within each group, individuals were ranked (1–6) based on their relative size, with the largest being ranked 1. It was possible to recognize group members ($n = 248$, in 71 groups) on the basis of natural variation in their markings (Buston 2003c). Individuals did not move between groups (Buston 2003a). Reproduction was monitored every 1–2 days for 296 days, from February 7th to December 5th 1997, and by the end of the study, 64 of the 71 groups had laid eggs.

COMPONENTS OF REPRODUCTIVE SUCCESS

Four components of reproductive success were measured. Reproduction was readily detectable: in the days before spawning, the male spent much of his time preparing a nest site, and in the days after spawning, he spent much of his time tending the eggs. The age of the eggs was determined by their colour: 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. The eggs hatched after 7 days. To get a good estimate of the number of eggs laid, every clutch of eggs was filmed for 1 min on day 1 or 2 – a clutch was considered laid if the female was no longer laying eggs. To get a good estimate of the number of eggs hatched, every clutch of eggs was

filmed for 1 min on day 5 or 6 – a clutch was considered hatched if it reached day 5 or 6. Eggs were filmed using Hi8 metal evaporated videotape and a Sony CCDTR700 8-mm video camera, along with an Amphibico Dive Buddy III underwater housing with dome port. The number of eggs laid and hatched was counted from frozen frames of tape on a high-resolution screen. This monitoring yielded data on (i) the ‘probability of laying eggs’ (0 or 1), on any given day; (ii) the ‘number of eggs laid’, given that eggs were laid; (iii) the ‘probability of hatching eggs’ (0 or 1), given that eggs were laid; and (iv) the ‘proportion of eggs hatched’, given that eggs were hatched. Also, the monitoring yielded data on the ‘total number of eggs hatched’ per pair per lunar month.

INDIVIDUAL FACTORS

Four measures of individual phenotype were made, for both females and males. In January 1997, all fish were captured using hand nets and taken to the surface in plastic bags. There, the initial ‘standard length’ (SL) of each individual was measured to 0.1 mm, using callipers. Also, the initial ‘mass’ of individuals was measured to 0.1 g, by placing them in a vessel of water on a micro-balance. In December 1997, all surviving fish were recaptured and remeasured, which permitted estimation of two measures of growth: ‘change in SL’, measured to 0.1 mm; and ‘change in mass’, measured to 0.1 g. We include both length and mass in our analyses, even though they are highly correlated, because they represent different things.

Two measures were made at the scale of the pair. The ordinal number of each clutch was measured over the entire year (starting at one), and this number was called ‘year clutch’. Year clutch indicates whether a clutch is a pair’s first, second, third or *n*th clutch for the given study period. Also, the ordinal number of each clutch was measured over each lunar month (starting at one), and this number was called ‘month clutch’. Month clutch indicates whether the clutch is a pair’s first, second or third clutch for a given lunar month. Together, these measurements yielded 10 variables representing characteristics of individuals and pairs that were predicted to influence the reproductive success of dominant pairs.

SOCIO-ECOLOGICAL FACTORS

Four measures of individual phenotype were made for nonbreeders, ranks 3–5: initial ‘SL’, initial ‘mass’, ‘change in SL’ and ‘change in mass’. Also, four factors that represent attributes of groups and the anemone were measured. The ‘number of nonbreeders’ present in the group was measured every other day. An experiment was conducted, in which nonbreeders were removed from 14 of anemones, so the categorical variables ‘treatment’ (manipulated or control) and ‘timing’ (before or after) were included in the analyses. The ‘anemone diameter’ was measured to the nearest 5 cm, using a measuring tape. These measurements yielded sixteen socio-ecological variables that were predicted to influence the reproductive success of dominant pairs.

SPATIO-TEMPORAL FACTORS

Two spatial and two temporal factors were measured. The spatial variable ‘reef’ identified the reef on which the group was located. Also, ‘depth’ of each anemone was measured to 0.1 m, using a dive computer. The temporal variable ‘lunar month’ was measured with month 1 being the first month of monitoring. Also, ‘lunar day’ was measured with day 1 being the first day after each new

moon. These measurements yielded four spatio-temporal variables that were predicted to influence reproductive success of dominant pairs.

STATISTICAL/MACHINE LEARNING ANALYSES

All analyses were conducted using BRTs (Friedman, Hastie & Tibshirani 2000; Schapire 2003; Elith *et al.* 2006; Elith, Leathwick & Hastie 2008; Leathwick *et al.* 2006, 2008; De’ath 2007). All BRTs were fitted in R (R Development Core Team 2006 version 2.3-1), using *gbm* package version 1.5-7 (Ridgeway 2006) plus custom code that is available online (Elith, Leathwick & Hastie 2008). BRTs have their origins in machine learning, but can be considered an advanced form of regression (Schapire 2003; Friedman, Hastie & Tibshirani 2000). In contrast to standard regression methods that produce a single predictive model, BRTs fit multiple simple models and combine them for prediction, thereby improving predictive performance. BRTs get their name from the two algorithms on which they are based: regression trees and boosting.

Regression trees partition predictor space into rectangles, using a set of rules to identify regions that have the most homogenous responses to predictors, and fitting the mean response for observations in each region (see Breiman *et al.* 1984; De’ath & Fabricius 2000; Hastie, Tibshirani & Friedman 2001; and Elith, Leathwick & Hastie 2008 for more detail). The pros of regression trees are that they can accommodate any type of variable (numeric, binary, categorical, also missing and non-independent data), and they successfully identify important predictor variables; the cons of regression trees are that they have difficulty modelling smooth functions, and they are not particularly robust to changes in training data (Elith, Leathwick & Hastie 2008).

Boosting helps to tackle the weaknesses of single regression trees. Boosting improves model accuracy, by searching for many rough prediction rules rather than the single most accurate prediction rule (Schapire 2003). There are several approaches to fitting a boosted model. Here, stochastic gradient descent was employed, and this can be thought of as a forward stagewise process, in which each successive tree is fitted to the residuals of the set so far selected. By shrinking the contribution of each tree and averaging across the final selected set, boosting generates a final model that is more robust than a single regression tree model and enables curvilinear functions to be modelled (Elith, Leathwick & Hastie 2008).

BRT analyses require the user to adjust two important parameters: the ‘learning rate’ and ‘tree complexity’. The learning rate determines the contribution of each tree to the growing model. The tree complexity controls whether interactions are fitted: a value of one fits an additive model, a value of two fits a model with two-way interactions and so on. Elith, Leathwick & Hastie (2008) provide more details of these parameters and provide rules of thumb for selecting appropriate settings. Here, learning rates that were slow enough (0.005–0.0005) to build models that estimate responses reliably and a tree complexity that was high enough (5) to build models with complex interactions were selected.

When developing BRT models, cross-validation (CV) can be used for model development and evaluation. CV provides a means for progressively testing the developing model on withheld portions of the data, ensuring the model that is finally fitted is general enough to predict well to held out data (see Elith, Leathwick & Hastie 2008 for explanation of its use in BRTs). The CV can be organized to deal with inherent structure in the data (e.g. Fabricius & De’ath 2008). Here, we used 10-fold CV and retained the natural structure in the data by keeping all data for any one anemone in the same fold (*c.* 7 anemones

per fold). This enabled us to make and test predictions about anemones not used in model fitting ('left-out' data).

Given the structure of the data, in traditional statistical models, it is necessary to enter group identification number as a random effect, to control for the lack of independence among multiple measures of reproduction from the same group (Buston 2004a). Our CV set-up is an established method for dealing with random effects in BRTs (Fabricius & De'ath 2008). To explore whether it successfully fitted a model of the response that accounted for the majority of variation between anemones, we tested the relationship between group ID and the residuals of the models using methods suggested by Wood (2006, section 6.5). In two of five cases, there was a relationship between group ID and the residuals of the model. The potential meaning of the group ID effect is discussed in more detail later.

The performance of all models was assessed on the training data and on predictions to test data (anemones that were withheld during CV), enabling us to evaluate our ability to explain observed data and predict left-out data, respectively. One or two measures of performance were calculated for each model, as appropriate (Ferrier & Watson 1997): the predictive deviance expressed as a percentage of the null deviance and the discrimination ability measured as the area under the receiver operator characteristic curve (AUC). While there are no *P* values in BRTs, the relative influence of individual predictors is estimated based on both how often the predictor is selected and the improvement to the model as the result of selection. Relative influence of predictors is then scaled so that the sum adds up to 100%, and here, we focus on predictors with a relative influence of more than 5%.

Visualization of fitted functions is achieved using partial dependence plots, which show the effect of a focal predictor on the response controlling for the average effect of all other variables in the model. Although partial responses from one tree yield step functions, adding information from additional trees can create complex curvilinear functions. Interactions between predictors are modelled automatically, because the structure of a tree means that the response to one predictor variable depends on values of predictors higher in the tree. Here, the partial dependence plots for the six most important predictors of reproductive success are presented, and interactions are explained in the text.

The effect of multiple predictors on each of the four components of reproductive success was investigated using BRTs. Four separate analyses were conducted because the bimodal distribution of the number of eggs laid on any given day, with a zero and nonzero mode, indicated that different processes might be causing the binary and numeric response. The same applied to the proportion of eggs hatched given that they are laid. Models for probability of laying or hatching assumed binomial errors; for the number of eggs laid, poisson errors; and for the proportion of eggs hatched, Gaussian errors on arc-sine-transformed percentages. The aim of these analyses was to identify the major predictors of individual components of reproductive success.

Results

PROBABILITY OF LAYING EGGS

The probability that dominant pairs laid eggs on any given day was 0.04 ± 0.02 (mean \pm SD). There were six important predictors of the probability that the dominant pair laid eggs on any given day: lunar day, presence of eggs, lunar

month, change in male SL, year clutch and month clutch (Table 1). The model explained 24% of the deviance using training data, which tells us how good the model is at explaining observed data; the model explained 15% of the deviance using CV, which tells us how good the model is at predicting left-out data. The area under the receiver operating characteristic curve was 0.88 in training and 0.82 in CV. This measure can be interpreted as the probability that when we randomly pick one laying event and one nonlaying event, the classifier will assign a higher score to the laying event than the nonlaying event.

The partial response plots (Fig. 1) indicate that dominant pairs are more likely to lay eggs around lunar day 19 than at other times, they do not lay eggs if they already have eggs, and they are more likely to lay a first clutch than they are to lay a second or third clutch in a given lunar month. They indicate that the probability of laying eggs fluctuates with lunar month. Finally, the likelihood of laying eggs decreases as change in male SL increases and increases with each additional clutch that that a pair lays.

There was one important interaction between predictors of the probability of laying eggs. The relationship between lunar date and the probability of laying eggs was dependent on month clutch, i.e. dependent on whether it was the first, second or third clutch of the month. The probability of laying the first clutch peaked around lunar day 14–15, the second clutch around day 18–19 and the third clutch around day 22–23. [There was also a set of trivial interactions between the predictor 'eggs present/absent' and all other predictors: when eggs were present, there was a zero probability of laying eggs; when eggs were absent, the responses were those seen in the partial dependence plot (Fig. 1)].

NUMBER OF EGGS LAID

The number of eggs that dominant pairs laid, given that they laid, was 324 ± 153 (mean \pm SD) and ranged from 1 to 878. Six variables were important predictors of the number of eggs laid by the dominant pair, given that they laid eggs: change in male SL, change in female mass, year clutch, female SL, male SL and lunar month (Table 1). The model explained 62% of the deviance using training data (explanation of observed data) and 15% of the deviance using CV (prediction of left-out data).

The partial response plots (Fig. 2) indicate that dominant pairs lay fewer eggs as change in male SL increases, but more eggs with each additional clutch that a pair lays. Also, the number of eggs laid is positively related to change in female mass and initial female SL. Finally, the number of eggs laid is positively related to initial male SL and varies with lunar month. There were no important interactions among predictors of the number of eggs laid.

Subsequent investigation of the residuals of this model revealed that they were related to group ID. This means that there was an effect of group ID on the number of eggs laid, over and above all of the other effects measured. This result

Table 1. Predictors of the reproductive success in dominant pairs of clownfish

Category	Predictor	Relative contribution (%)				
		Probability of laying eggs	Number of eggs laid	Probability of hatching eggs	Proportion of eggs hatched	Total eggs hatched
Characteristics of individuals and pairs	F SL	0.80	7.40	0.26	1.14	4.20
	F mass	0.83	3.33	0.13	0.52	2.84
	F change in SL	3.39	3.81	8.85	2.13	3.11
	F change in mass	1.84	13.64	6.39	0.51	10.17
	M SL	1.15	6.40	1.56	1.04	2.23
	M mass	0.98	1.36	1.07	0.63	1.07
	M change in SL	8.04	18.05	1.39	15.46	27.79
	M change in mass	2.68	1.29	3.41	3.74	1.06
	Year clutch	7.30	10.52	1.96	1.76	13.42
Month clutch	5.77	0.77	0.44	0.82	–	
Characteristics of groups and territories	R3 SL	2.00	0.79	0.10	1.83	2.47
	R3 mass	0.75	0.71	0.04	1.47	1.34
	R3 change in SL	0.84	3.36	0.50	1.50	1.18
	R3 change in mass	0.56	4.96	0.20	0.46	3.57
	R4 SL	0.49	1.77	0.12	1.11	1.42
	R4 mass	0.35	0.39	0.05	0.56	0.96
	R4 change in SL	0.83	1.16	0.00	0.16	0.51
	R4 change in mass	0.40	1.17	0.02	0.06	2.45
	R5 SL	0.18	0.08	0.02	0.20	0.48
	R5 mass	0.10	0.01	0.02	0.01	0.19
	R5 change in SL	0.06	0.05	0.01	0.10	0.02
	R5 change in mass	0.06	0.01	0.00	0.09	0.02
	Nonbreeder number	0.33	0.10	0.03	0.06	0.08
	Control/Experiment	0.47	0.04	0.00	0.21	1.06
	Before/After	0.02	0.09	0.01	0.06	0.05
Anemone diameter	1.38	2.99	0.72	10.53	1.38	
Spatio-temporal variables	Depth	2.47	3.82	0.33	10.29	7.70
	Reef	0.43	4.68	0.02	0.16	1.32
	Lunar day	30.13	1.79	0.53	3.11	–
	Lunar month	9.16	5.45	4.52	19.89	7.86
Other	Eggs present/absent	16.21	–	–	–	–
	Number of eggs laid	–	–	67.32	20.40	–

SL, standard length.

Numbers summarize the relative contributions of predictor variables for boosted regression tree models developed to predict four components of reproductive success and overall reproductive success. Numbers in bold highlight the variables that contribute more than 5% to a given model. These variables were considered important predictors of reproductive success (see Figures).

Buston (2004a) conducted an experiment in which nonbreeders were removed from 14 anemones at the end of lunar month 4. Control/Experiment is a categorical variable, indicating whether measures of reproductive success were from anemones destined to have nonbreeders removed. Before/After is a categorical variable, indicating whether measures of reproductive success came before or after the removal.

indicates that there is unmeasured variation in dominant pairs, their territories or the interaction between dominant pairs and their territories that is related to the number of eggs laid per clutch.

PROBABILITY OF HATCHING EGGS

The probability that dominant pairs hatched eggs, given that they laid, was 0.65 ± 0.31 (mean \pm SD). Three variables were important predictors of the probability that the dominant pair hatched eggs, given that they laid them: the number of eggs laid, change in female SL and change in female mass (Table 1). The model explained 48% of the deviance using training data (explanation of observed data) and 31% of the deviance using CV (prediction of left-out data). The area

under the receiver operating characteristic curve was 0.93 in training and 0.79 in CV.

The partial response plots (Fig. 3) for these variables indicate that dominants are unlikely to hatch eggs when the number of eggs laid is particularly small (less than ≈ 100 eggs). They indicate that the pair is less likely to hatch eggs as female change in SL and change in mass increases. There were no important interactions among predictors of the probability of hatching eggs.

PROPORTION OF EGGS HATCHED

The proportion of eggs that dominant pairs hatched, given that they hatched, was 0.87 ± 0.16 (mean \pm SD). Five variables were predictors of the proportion of eggs hatched by

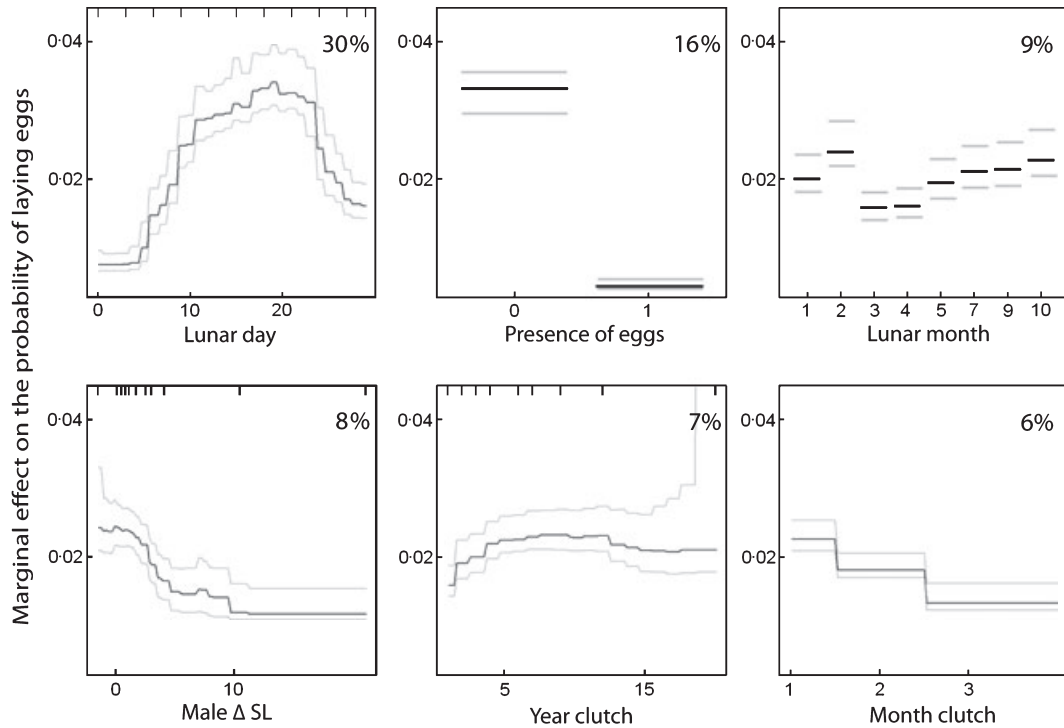


Fig. 1. Probability of laying eggs. Partial dependence plots for the six most influential variables that predict the probability that dominant pairs lay eggs on any given day. Fitted lines represent the mean estimate (black) and 95% confidence intervals (grey) based on 500 bootstrap replicates. Rug plots at inside top of plots show the distribution of data, in deciles, of the variable on the X -axis.

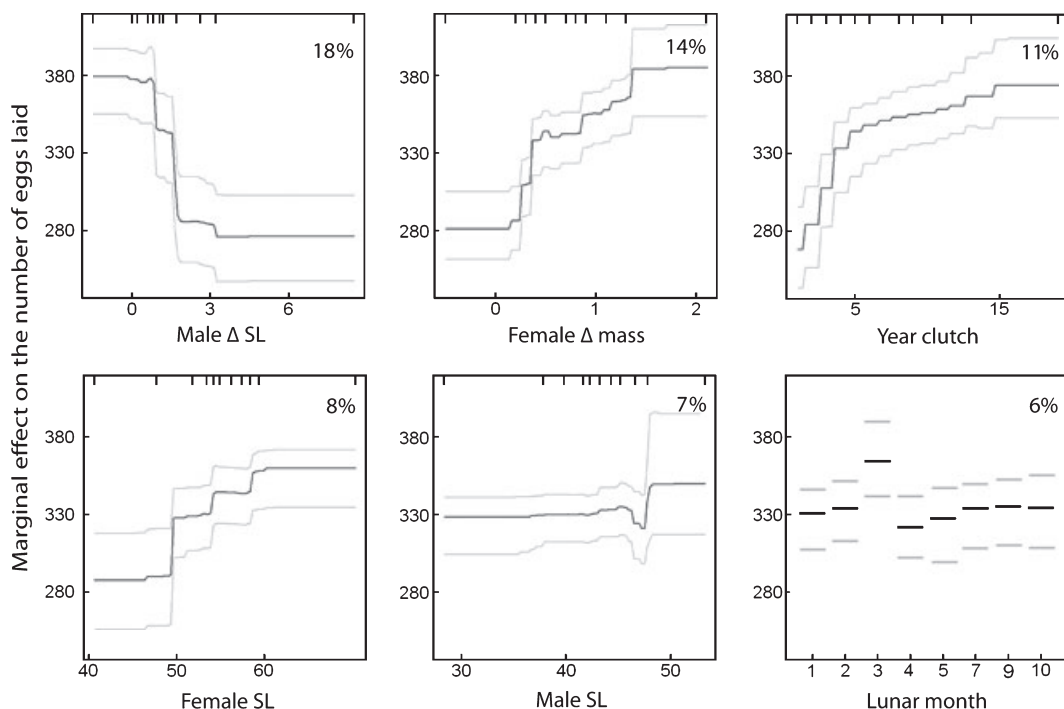


Fig. 2. Number of eggs laid. Partial dependence plots for the six most influential variables that predict the number of eggs that dominant pairs lay, given that they lay eggs. Fitted lines represent the mean estimate (black) and 95% confidence intervals (grey) based on 500 bootstrap replicates. Rug plots at inside top of plots show the distribution of data, in deciles, of the variable on the X -axis.

the dominant pair: the number of eggs laid, lunar month, change in male SL, anemone diameter and depth (Table 1). The model explained 12% of the deviance using training data (explanation of observed data) but none of the deviance on

the left-out data. Given that the model is not useful for prediction, we will not interpret it further. We note, however, that the partial responses (Fig. 4) may be real patterns in a noisy data set.

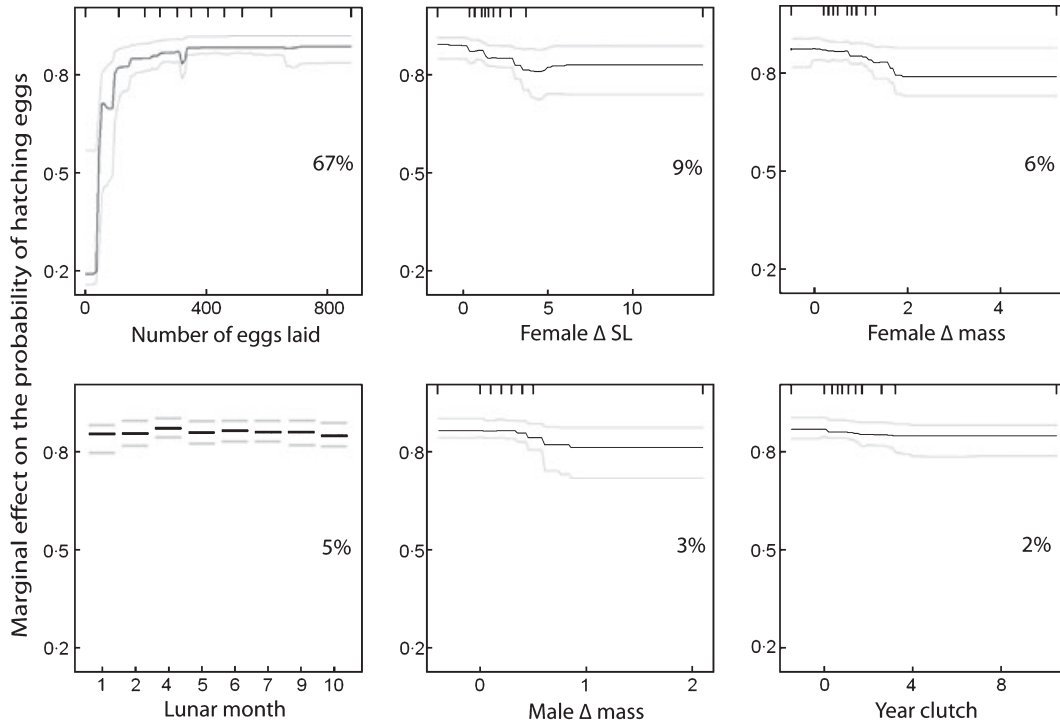


Fig. 3. Probability of hatching eggs. Partial dependence plots for the six most influential variables that predict the probability that dominant pairs hatch at least one of the eggs they lay. Fitted lines represent the mean estimate (black) and 95% confidence intervals (grey) based on 500 bootstrap replicates. Rug plots at inside top of plots show the distribution of data, in deciles, of the variable on the X-axis.

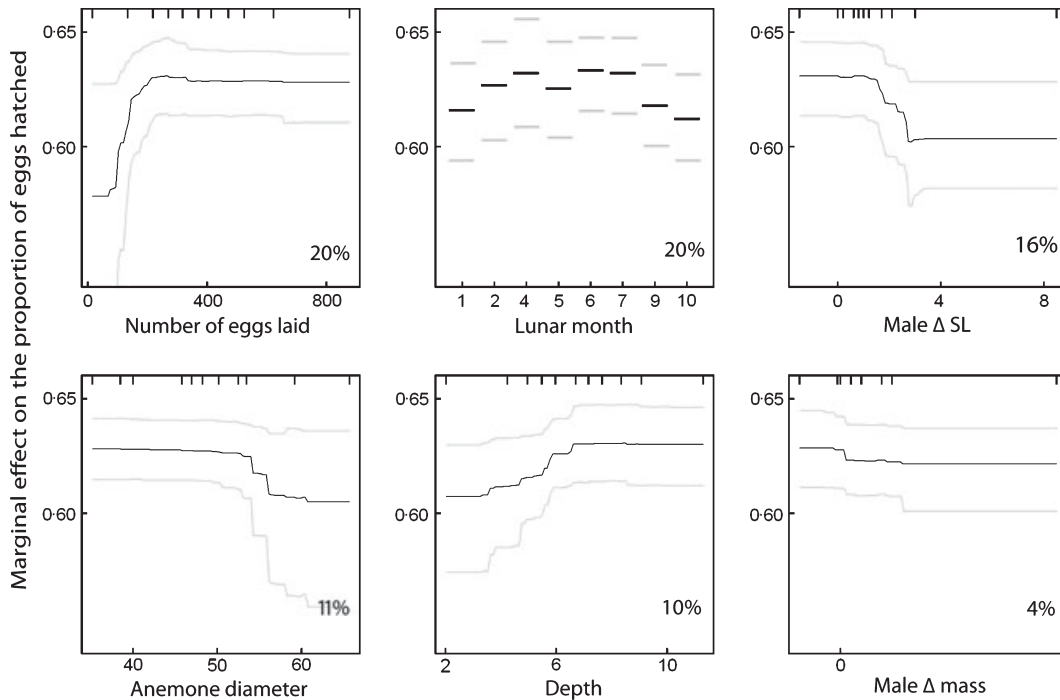


Fig. 4. Proportion of eggs hatched. Partial dependence plots for the six most influential variables that predict the proportion of eggs that dominant pairs hatch, given that they hatch eggs. Fitted lines represent the mean estimate (black) and 95% confidence intervals (grey) based on 500 bootstrap replicates. Rug plots show the distribution of data, in deciles, of the variable on the X-axis.

TOTAL NUMBER OF EGGS HATCHED

Five variables were important predictors of the total number of eggs hatched per dominant pair per lunar

month: change in male SL, year clutch, change in female mass, lunar month and depth (Table 1). The model explained 51% of the deviance using training data (explanation of observed data); the model explained

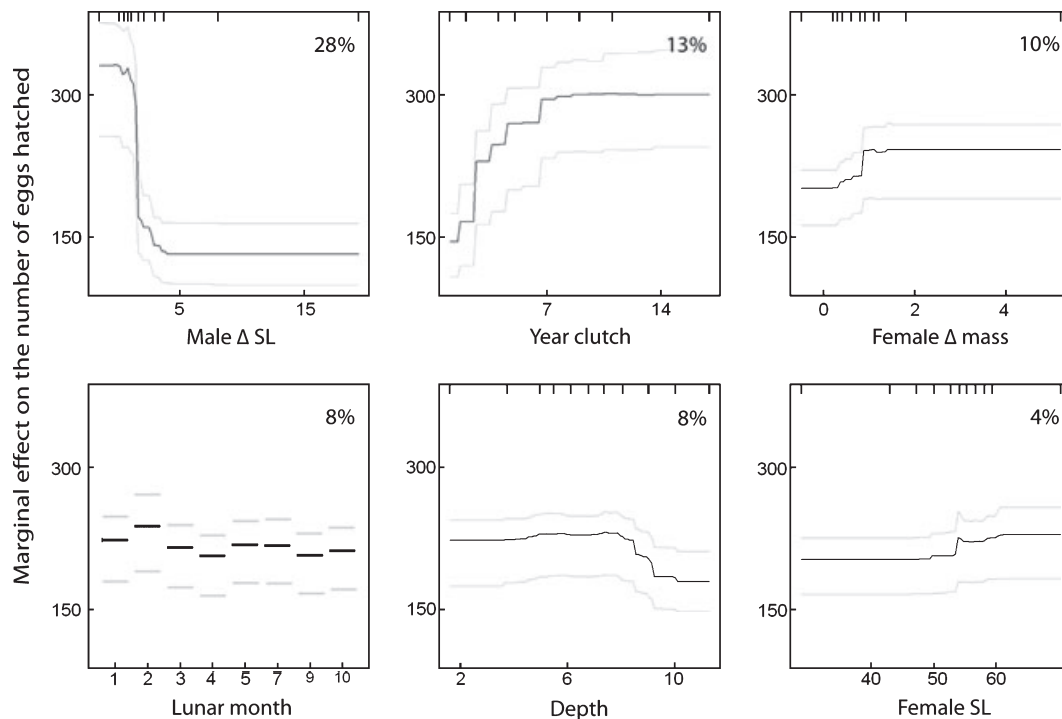


Fig. 5. Total number of eggs hatched. Partial dependence plots for the six most influential variables that predict the total number of eggs that dominant pairs hatch per lunar month. Fitted lines represent the mean estimate (black) and 95% confidence intervals (grey) based on 500 bootstrap replicates. Rug plots at inside top of plots show the distribution of data, in deciles, of the variable on the X-axis.

19% of the deviance using CV (prediction of left-out data).

The partial responses (Fig. 5) for the six major predictors indicate that the dominant pair hatch more eggs per month as change in male SL decreases, but more eggs with each additional clutch that a pair lays (see also Figs 1 and 2). Pairs hatch more eggs per month as change in female mass increases and as initial female SL increases (see also Fig. 2). Finally, the number of eggs hatched varies with depth (see also Fig. 4) and from 1 month to the next (see also Figs 1 and 4).

There was one important interaction between predictors of the total number of eggs hatched per month: the effect of change in female mass on the number of eggs hatched varied with the effect of change in male SL and vice versa. Specifically, when males changed length by more than a couple of millimetres during the year, the number of eggs hatched per month was very low, and there was little or no effect of change in female mass on the number of eggs hatched. In contrast, when males changed in length less than a couple of millimetres during the year, the number of eggs hatched was high, and change in female mass was positively related to the number of eggs hatched.

Subsequent investigation of the residuals of this model revealed that they were related to group ID. This means that there was an effect of group ID on the total number of eggs hatched, over and above all of the other effects measured. Likely, this is because of the effect of group ID on the number of eggs laid. This result indicates that there is unmeasured variation in dominant pairs, their territories or the interac-

tion between dominant pairs and their territories that is related to the total number of eggs hatched.

Discussion

We investigated the effects of individual, social, ecological and spatio-temporal factors on four components of reproductive success of dominant pairs of clown anemonefish. Our analyses indicate that change in male length is negatively related, and year clutch is positively related to the probability of laying eggs (Fig. 1) and the number of eggs laid per clutch (Fig. 2). These effects, taken together, are the primary determinants of the total number of eggs hatched by pairs (Fig. 5, Table 1). Also, our analyses indicate that change in female mass and initial female SL are both positively related to the number of eggs laid per clutch (Fig. 2). These effects, taken together, can be considered the secondary determinants of the total number of eggs hatched by pairs (Fig. 5, Table 1). Our analyses indicate that there is no important effect of the number of nonbreeders, nonbreeder removal or nonbreeder phenotype on any component of the reproductive success of pairs (Figs 1–5, Table 1), confirming and extending the results of a previous analysis (Buston 2004a). Finally, our analyses indicate that there are spatial (depth) and temporal (lunar month) effects on the reproductive success of pairs (Fig. 5, Table 1), but that these are weak relative to the effect of traits of individuals. This study provides the most complete picture to date of the factors that predict anemonefish reproductive success.

BOOSTED REGRESSION TREES

In addition, this study illustrates the utility of BRTs for analysis of complex behavioural and evolutionary ecology data. The potential contribution of BRTs to the field should be assessed based on (i) how much additional insight into predictors they make and (ii) how many fewer assumptions they make, when compared to more traditional analyses. Buston (2004a) conducted a mixed model analysis investigating the effect of multiple factors on the total number of eggs hatched by pairs each lunar month, equivalent to the final analysis presented here. That mixed model indicated that male growth was negatively related, and nonbreeder removal was not related, to the total number of eggs hatched (Buston 2004a). Thus, BRTs are identifying predictors with large effects and predictors with small effects, in a manner consistent with mixed model analyses. BRTs, however, seem to do better when it comes in identifying predictors with subtler, intermediate, effects (contrast to Buston 2004a); BRTs also seem to do better when it comes to disentangling the effects of multiple correlated variables (contrast to Mitchell 2003). Perhaps the most important benefits of BRTs are that they can accommodate any type of variable and that they enable investigators to model complex curvilinear relationships (e.g. Fig. 5) and interactions without making assumptions regarding the nature of those relationships.

PRIMARY DETERMINANTS OF REPRODUCTIVE SUCCESS

The primary determinants of reproductive success of dominant pairs of *A. percula* are (i) change in male SL and (ii) year clutch. The negative relationship between change in male SL and reproductive success likely is indicative of a trade-off between male growth and parental investment. Such a trade-off is common in fishes although the underlying mechanism of the trade-off, be it a reduction in energy intake, an increase in energy expenditure, or reallocation of energy, varies (Helfman *et al.* 2009). The positive relationship between year clutch and reproductive success has a subtle explanation. This relationship does not simply indicate that pairs that hatched many eggs in the first months of the study continued to hatch many eggs in later months. Rather, the relationship indicates that pairs hatched more eggs per month with each additional clutch that they laid. It seems reasonable to suggest that the variable 'year clutch' can be thought of as 'breeding experience' – the more experience pairs have, the more successful they become. It is likely that the true effect of breeding experience is stronger than that documented here because, in this data set, the first clutch laid by a pair represents the true first clutch for some but the *n*th clutch for others. Such an effect of breeding experience has not been quantified in fishes in the field before, even though aquarists are aware of the effect, and it has been documented in other taxa (Forslund & Pärt 1995). We consider the effects of change in male SL and breeding experience together because we believe that they might be related: in the field, following the death or experimental removal of a male, the new replace-

ment male grows a lot, and the newly formed pair has no breeding experience (Buston 2003b, 2004a). It will be interesting to investigate the mechanism underlying the effects of male growth and breeding experience on reproductive success in more detail in the future.

SECONDARY DETERMINANTS OF REPRODUCTIVE SUCCESS

The secondary determinants of reproductive success of dominant pairs of *A. percula* are (i) change in female mass and (ii) female SL. The positive relationship between female SL and reproductive success is consistent with results of other studies of anemonefishes (Mitchell 2003; Green & McCormick 2005a). Such a relationship is common in fishes and likely reflects a capacity constraint – large females can carry more eggs (Helfman *et al.* 2009). The positive relationship between female change in mass and reproductive success is more surprising, given that one might expect a trade-off between growth and reproduction (Bell & Koufopanou 1986; Partridge & Harvey 1988; Reznick, Nunney & Tessier 2000; Roff 2002). The simplest hypotheses for this positive association are that variation in female quality or territory quality means that some individuals are able to both grow and reproduce successfully (van Noordwijk & De Jong 1986; De Jong & van Noordwijk 1992; Malausa, Guillemaud & Lapchin 2005). Interestingly, in this population, female size and growth are positively related to group size and anemone size also (Fautin 1992; Elliott & Mariscal 2001; Buston 2002; Buston & Cant 2006). The observation that the positive correlations between traits extend beyond the female suggests that variation in territory quality (either anemone quality or site quality), rather than variation in female quality, is the more likely cause of this effect. If variation in territory quality is indeed the cause of the positive relationship between female growth and reproductive success, then this suggests that there is a fairly strong 'silver-spoon effect' in *A. percula*, whereby individuals that are fortunate enough to recruit into good territories reap long-term rewards in terms of growth, size and reproductive success (Grafen 1988; Madsen & Shine 2000; van de Pol *et al.* 2006).

THE EVOLUTION OF REPRODUCTIVE STRATEGIES

Having identified the primary and secondary determinants of reproductive success, we now turn to consider whether our results can shed any light on the evolution of the most notable of clownfish reproductive strategies: sex change. Fricke & Fricke (1977) were the first to describe sex change in *Amphiprion*, yet hypotheses for why the trait is maintained by natural selection lack empirical support. The size advantage hypothesis (SAH) is widely used to understand the evolution of sex change (Ghiselin 1969; Warner 1975; Charnov 1982). The SAH predicts that the sexual tactic adopted by individuals will depend on the relationship between body size and reproductive value, which may in turn be influenced by mating system or social context (Warner 1984, 1988; Munday, Buston

& Warner 2006). In a monogamous system, the SAH predicts that if expected offspring production as a female increases with body size more strongly than does expected offspring production as a male, then selection will favour the larger of the pair adopting the female tactic and the smaller of the pair adopting the male tactic (Warner 1984; Buston 2002; Munday, Buston & Warner 2006). These are the very relationships between offspring production, body size and sexual tactic that we have uncovered here (Fig. 5, Table 1) and, given that the mortality rates associated with male and female tactics are the same (Buston 2003c), this is sufficient to explain the maintenance of male first sex change by selection in this species. Indeed, we can estimate that both individuals increase their short-term reproductive success by *c.* 10% by coordinating over reproductive tactics in this way. To our knowledge, this is the first empirical demonstration of the proposed selective advantage of male first sex change in *Amphiprion*. This highlights the power of long-term studies of marked individuals in conjunction with statistical learning techniques to provide new biological insights.

Conclusion

This study culminates a series of articles that have investigated various aspects of the behavioural and population ecology of *A. percula*, in Madang Lagoon, Papua New Guinea (Buston 2002). Our results support the conclusion that subordinate nonbreeders have neither positive nor negative effects on the short-term reproductive success of dominant pairs (Buston 2004a). More compellingly, our results suggest that traits of the male, female and pair's interaction are the most important drivers of short-term reproductive success. Future studies might investigate whether an effect of subordinates on the dominant pairs' reproductive success is hidden by adjustments in maternal or paternal investment (Green & McCormick 2005a; Russell *et al.* 2007; Taborsky, Skubic & Brintjes 2007; Russell & Lumaa 2009) or whether subordinates influence the dominant pairs' growth, size and resultant reproductive success, via effects on the anemone, i.e. whether there is a group augmentation effect (Kokko, Johnstone & Clutton-Brock 2001; Clutton-Brock 2002; Porat & Chadwick-Furman 2004; Holbrook & Schmitt 2005). We suggest, however, that it will be most profitable to use experiments to investigate the cause and effect of relationships demonstrated here or field studies to investigate how the relationships demonstrated here translate into the number of offspring that survive to breed following dispersal (Jones, Planes & Thorrold 2005; Planes, Jones & Thorrold 2009; P.M. Buston, G.P. Jones, S. Planes & S.R. Thorrold, unpublished data).

Acknowledgements

We thank John Drake, Bill Langford, John Leathwick, Dragos Margineantu, Andrew Russell, Philip Stephens and Daniel Stouffer for helpful comments and discussion; Stephen Emlen, Paul Sherman, Andrew Bass, Amy McCune and Kern Reeve for consistent advice and support; John Mizeu, Mike Black, Claire Norris and Mike Moore for field assistance; the staff at the Christensen Research Institute and Jais Aben Resort for logistical support; and the land-

owners of Riwo village, the Madang Provincial Government and Papua New Guinea Government for permission to work in Madang Lagoon. Field portion of this project is a product of Buston's Ph.D. dissertation. Financial support for the field portion came from Diane Christensen and the Christensen Fund; National Science Foundation, Dissertation Improvement Grant; Andrew W. Mellon Student Research Grant; Cornell, Graduate Research Travel Grant; Cornell and National Chapters of Sigma Xi, Grants-in-Aid of Research; International Women's Fishing Association, Scholarship; Percy Sladen Memorial Fund; Cornell, Department of Neurobiology and Behaviour and Department of Ecology and Evolutionary Biology. Analytical portion of the project is a product of the Ecological Machine Learning Working Group at NCEAS, a center funded by NSF grant #EF-0553768, the University of California and the Santa Barbara campus (USA). JE was supported by Australian Research Council grants DP0772671 and FT0991640. PB was supported by the Department of Biology at Boston University.

References

- Bell, G. & Koufopanou, V. (1986) The cost of reproduction. *Oxford Surveys in Evolutionary Biology*, **3**, 83–131.
- Breiman, L., Friedman, J.H., Olshen, R.A. & Stone, C.J. (1984) *Classification and Regression Trees*. Wadsworth International Group, Belmont, CA, USA.
- Brouwer, L., Heg, D. & Taborsky, M. (2005) Experimental evidence for helper effects in a cooperatively breeding cichlid. *Behavioral Ecology*, **16**, 667–673.
- Brown, J.L., Brown, E.R., Brown, S.D. & Dow, D.D. (1982) Helpers: effects of experimental removal on reproductive success. *Science*, **215**, 421–422.
- Buston, P.M. (2002) Group structure of the clown anemonefish *Amphiprion percula*. PhD dissertation, Cornell University, USA.
- Buston, P.M. (2003a) Forcible eviction and prevention of recruitment in the clown anemonefish. *Behavioral Ecology*, **14**, 576–582.
- Buston, P.M. (2003b) Size and growth modification in clownfish. *Nature*, **424**, 145–146.
- Buston, P.M. (2003c) Mortality is associated with social rank in the clown anemonefish (*Amphiprion percula*). *Marine Biology*, **143**, 811–815.
- Buston, P.M. (2004a) Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clownfish *Amphiprion percula*. *Behavioural Ecology and Sociobiology*, **57**, 23–31.
- Buston, P.M. (2004b) Territory inheritance in clownfish. *Proceedings of the Royal Society of London, Series B (Suppl.)*, **271**, S252–S254.
- Buston, P.M. & Cant, M.A. (2006) A new perspective on size hierarchies in nature: patterns, causes, and consequences. *Oecologia*, **149**, 362–372.
- Buston, P.M., Bogdanowicz, S.M., Wong, A. & Harrison, R.G. (2007) Are clownfish groups composed of close relatives? Analysis of microsatellite DNA variation in *Amphiprion percula*. *Molecular Ecology*, **16**, 3671–3678.
- Charnov, E.L. (1982) *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.
- Clutton-Brock, T.H. (2002) Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, **296**, 69–72.
- Cockburn, A. (1998) Evolution of helping behaviour in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, **29**, 141–177.
- Cockburn, A., Sims, R.A., Osmond, H.J., Green, D.J., Double, M.C. & Mulder, R.A. (2008) Can we measure the benefits of help in cooperatively breeding birds: the case of superb fairy-wrens *Malurus cyaneus*? *Journal of Animal Ecology*, **77**, 430–438.
- De Jong, G. & van Noordwijk, A.J. (1992) Acquisition and allocation of resources: genetic (co)variances, selection and life histories. *American Naturalist*, **139**, 749–770.
- De'ath, G. (2007) Boosted trees for ecological modeling and prediction. *Ecology*, **88**, 243–251.
- De'ath, G. & Fabricius, K.E. (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178–3192.
- Dickinson, J. & Hatchwell, B.J. (2004) Fitness consequences of helping. *Ecology and the Evolution of Cooperative Breeding in Birds* (eds W. Koenig & J. Dickinson), pp. 48–66. Cambridge University Press, Cambridge.
- Elith, J., Leathwick, J.R. & Hastie, T. (2008) A working guide to boosted regression trees. *Journal of Animal Ecology*, **77**, 802–813.
- Elith, J., Graham, C.H., Anderson, R.P., Dudik, M., Ferrier, S., Guisan, A. *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.
- Elliott, J.K., Elliott, J.M. & Mariscal, R.N. (1995) Host selection, location, and association behaviors of anemonefishes in field settlement experiments. *Marine Biology*, **122**, 377–389.

- Elliott, J.K. & Mariscal, R.N. (2001) Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. *Marine Biology*, **138**, 23–36.
- Emlen, S.T. (1991) Evolution of cooperative breeding in birds and mammals. *Behavioural Ecology: An Evolutionary Approach* (eds J.R. Krebs & N.B. Davies), pp. 301–337. Blackwell Scientific, Oxford.
- Emlen, S.T. (1997) Predicting family dynamics in social vertebrates. *Behavioral Ecology: An Evolutionary Approach* (eds J.R. Krebs & N.B. Davies), pp. 228–253. Blackwell Publishing, Oxford.
- Emlen, S.T. & Wrege, P.H. (1991) Breeding biology of white-fronted bee-eaters at Nakuru: the influence of helpers on breeder fitness. *Journal of Animal Ecology*, **60**, 309–326.
- Fabricsius, K.E. & De'ath, G. (2008) Photosynthetic symbionts and energy supply determine octocoral biodiversity in coral reefs. *Ecology*, **89**, 3163–3173.
- Fautin, D.G. (1997) Anemonefish recruitment: the roles of order and chance. *Symbiosis*, **14**, 143–160.
- Ferrier, S. & Watson, G. (1997) An evaluation of the effectiveness of environmental surrogates and modelling techniques in predicting the distribution of biological diversity, pp. 1–184. Consultancy report prepared by the NSW National Parks and Wildlife Service for Department of Environment, Sport and Territories, Environment Australia, Canberra. <http://www.deh.gov.au/biodiversity/publications/technical/surrogates/>
- Forslund, P. & Pärt, T. (1995) Age and reproduction in birds: hypotheses and tests. *Trends in Ecology and Evolution*, **10**, 374–378.
- Fricke, H. & Fricke, S. (1977) Monogamy and sex change by aggressive dominance in a coral reef fish. *Nature*, **266**, 830–832.
- Friedman, J.H., Hastie, T. & Tibshirani, R. (2000) Additive logistic regression: a statistical view of boosting. *Annals of Statistics*, **28**, 337–407.
- Ghiselin, M.T. (1969) The evolution of hermaphroditism among animals. *The Quarterly Review of Biology*, **44**, 189–208.
- Grafen, A. (1988) On the uses of data on lifetime reproductive success. *Reproductive Success. Studies of Individual Variation in Contrasting Breeding Systems* (ed. T.H. Clutton-Brock), pp. 454–471. University of Chicago Press, Chicago.
- Green, B.S. & McCormick, M.I. (2005a) Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. *Marine Ecology Progress Series*, **289**, 263–272.
- Hastie, T., Tibshirani, R. & Friedman, J.H. (2001) *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. Springer-Verlag, New York.
- Helfman, G.S., Collette, B.B., Facey, D.E. & Bowen, B.W. (2009) *The Diversity of Fishes*. Blackwell Publishing, Oxford.
- Holbrook, S.J. & Schmitt, R.J. (2005) Growth, reproduction and survival of a tropical sea anemone (Actinaria): benefits of hosting anemonefish. *Coral Reefs*, **24**, 67–73.
- Jones, G.P., Planes, S. & Thorrold, S.R. (2005) Coral reef fish larvae settle close to home. *Current Biology*, **15**, 1314–1318.
- Kokko, H., Johnstone, R.A. & Clutton-Brock, T.H. (2001) The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society of London, Series B*, **268**, 187–196.
- Komdeur, J. (1994) Experimental evidence for helping and hindering by previous offspring in the cooperative-breeding Seychelles warbler *Acrocephalus sechellensis*. *Behavioural Ecology and Sociobiology*, **34**, 175–186.
- Leathwick, J.R., Elith, J., Francis, M.P., Hastie, T. & Taylor, P. (2006) Variation in demersal fish species richness in the ocean surrounding New Zealand: an analysis using boosted regression trees. *Marine Ecology Progress Series*, **321**, 267–281.
- Leathwick, J.R., Elith, J., Chadderton, W.L., Rowe, D. & Hastie, T. (2008) Dispersal, disturbance and the contrasting biogeographies of New Zealand's diadromous and non-diadromous fish species. *Journal of Biogeography*, **35**, 1481–1497.
- Madsen, T. & Shine, R. (2000) Silver spoons and snake body sizes: prey availability early in life influences long-term growth rates of free-ranging pythons. *Journal of Animal Ecology*, **69**, 952–958.
- Malaua, T., Guillemaud, T. & Lapchin, L. (2005) Combining genetic variation and phenotypic plasticity in tradeoff modeling. *Oikos*, **110**, 330–338.
- Mitchell, J.S. (2003) Social correlates of reproductive success in false clown anemonefish: subordinate group members do not pay-to-stay. *Evolutionary Ecology Research*, **5**, 89–104.
- Mitchell, J.S. (2005) Queue selection and switching by false clown anemonefish, *Amphiprion ocellaris*. *Animal Behaviour*, **69**, 643–652.
- Mitchell, J.S. & Dill, L.M. (2005) Why is group size correlated with the size of the host sea anemone in the false clown anemonefish? *Canadian Journal of Zoology*, **83**, 372–376.
- Mumme, R.L. (1992) Do helpers increase reproductive success? An experimental analysis in the Florida scrub jay. *Behavioural Ecology and Sociobiology*, **31**, 319–328.
- Munday, P.L., Buston, P.M. & Warner, R.R. (2006) Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology and Evolution*, **21**, 89–95.
- van Noordwijk, A.J. & De Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist*, **128**, 137–142.
- Partridge, L. & Harvey, P. (1988) The ecological context of life history evolution. *Science*, **241**, 1449–1455.
- 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 USA*, **106**, 5693–5697.
- van de Pol, M., Buinzeel, L.W., Heg, D., van der Jeugd, H.P. & Verhulst, S. (2006) A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). *Journal of Animal Ecology*, **71**, 616–626.
- Porat, D. & Chadwick-Furman, N.E. (2004) Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. *Hydrobiologia*, **530/531**, 513–520.
- Reznick, D., Nunney, L. & Tessier, A. (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology and Evolution*, **15**, 421–425.
- Ridgeway, G. (2006) Generalized Boosted Regression models. Documentation on the R package 'gbm', version 1-5-7. <http://www.i-pensieri.com/gregr/gbm.shtml>, accessed March 2008.
- Roff, D.A. (2002) *Life History Evolution*. Sinauer Associates, Inc., Sunderland, MA.
- Russell, A.F. & Lumaa, V. (2009) Maternal effects in cooperative breeders: from hymenopterans to humans. *Philosophical Transactions of the Royal Society, Series B*, **364**, 1143–1167.
- Russell, A.F., Langmore, N.E., Cockburn, A., Astheimer, L.B. & Kilner, R.M. (2007) Reduced egg investment can conceal helper effects in cooperatively breeding birds. *Science*, **317**, 941–944.
- Schapiro, R. (2003) The boosting approach to machine learning – an overview. *MSRI Workshop on Nonlinear Estimation and Classification, 2002* (eds D. D. Denison, M. H. Hansen, C. Holmes, B. Mallick & B. Yu), pp. 1–21. Springer, New York, USA.
- Taborsky, B., Skubic, E. & Bruintjes, R. (2007) Mothers adjust egg size to helper number in a cooperatively breeding cichlid. *Behavioral Ecology*, **18**, 652–657.
- Warner, R.R. (1975) The adaptive significance of sequential hermaphroditism in animals. *American Naturalist*, **109**, 61–82.
- Warner, R.R. (1984) Mating behavior and hermaphroditism in reef fishes. *American Scientist*, **72**, 128–136.
- Warner, R.R. (1988) Sex change and the size-advantage model. *Trends in Ecology and Evolution*, **3**, 133–136.
- Wood, S.N. (2006) *Generalised Additive Models: An Introduction with R*. Chapman and Hall/CRC Press, Boca Raton, FL.
- Wright, J. & Russell, A. (2008) How helpers help: disentangling ecological confounds from the benefits of cooperative breeding. *Journal of Animal Ecology*, **77**, 427–429.

Received 6 July 2010; accepted 28 December 2010

Handling Editor: Joseph Rasmussen