Genet-specific spawning patterns in *Acropora palmata*

Miller MW[1,*], Williams DE[1,2], Fisch J[3]

[1] Southeast Fisheries Science Center, National Marine Fisheries Service, 75 Virginia Beach Dr.

Miami FL 33149 USA

[2] Cooperative Institute for Marine and Atmospheric Science, University of Miami, 4600

Rickenbacker Cswy. Miami FL 33149 USA

[3] Division of Marine Biology and Ecology, University of Miami, 4600 Rickenbacker Cswy

Miami FL 33149 USA

*Corresponding author: Margaret.w.miller@noaa.gov

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Abstract

The broadcast spawning elkhorn coral, *Acropora palmata*, requires outcrossing among different genets for effective fertilization. Hence, a low density of genets in parts of its range emphasizes the need for precise synchrony among neighboring genets as sperm concentration dilutes rapidly in open-ocean conditions. We documented the genet-specific nightly occurrence of spawning of *Acropora palmata* over eight years in a depauperate population in the Florida Keys to better understand this potential reproductive hurdle. The observed population failed to spawn within the predicted monthly window (nights 2–6 after the full moon in August) in three of the eight years of observation; negligible spawning was observed in a fourth year. Moreover, genet-specific patterns are evident in that 1) certain genets have significantly greater odds of spawning overall and 2) certain genets predictably spawn on the earlier and others on the later lunar nights within the predicted window. Given the already low genet density in this population, this pattern implies a substantial degree of wasted reproductive effort and supports the hypothesis that depensatory factors are impairing recovery in this species.

Introduction

Many broadcast spawning coral species, including *Acropora* spp., are obligate outcrossers, requiring mixing of gametes from different genets for effective fertilization (Fukami et al. 2003; Baums et al. 2005). Precise synchrony of gamete release among genets is crucial to ensure successful larval production because broadcast gametes are viable over a period of only a few hours and sperm dilution rapidly degrades fertilization potential in ocean conditions (Oliver and Babcock 1992; Levitan et al. 2004). This required synchrony is expected to be particularly important in populations with low densities of individuals and genets. *Acropora palmata*, the

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39 threatened Caribbean elkhorn coral, is genotypically depauperate in some areas of its range and

40 significant declines in genotypic richness have been documented in the Florida Keys over very

41 short time scales (Williams et al. 2014). These characteristics confer a particular need for

42 spawning synchrony to allow for successful larval production in this population.

43   *Acropora* spp. have less tightly constrained spawning nights than other genera of

44 broadcasting corals. For example, in the Solitary Islands, Australia, Wilson and Harrison (2003)

45 document a much wider range of spawning nights among individual colonies (including across

46 months) for 12 *Acropora* spp. than among four mounding species. Among reports for Caribbean

47 broadcasters, Fogarty et al. (2012) compiled spawning observations indicating that *Acropora*

48 *palmata* spawns anywhere from night 1 to night 8 after the full moon (AFM), whereas Levitan et

49 al. (2004; 2011) indicate that *Orbicella* spp. in both Panama and in the Bahamas show significant

50 spawning on only two nights, within which individual corals did not show a preference for

51 spawning on one night or the other.

52   Currently, there are no published genet-specific records of spawning for Caribbean

53 *Acropora* spp. This study documented the lunar nights of spawning by individual genets of the

54 Caribbean elkhorn coral, *Acropora palmata*, in the Florida Keys over eight years to evaluate one

55 aspect of successful larval production in this imperiled population.

56

Methods

58   Spawning observations were made in the field during the expected *A. palmata* spawning

59 window (nights 2–6 AFM, (Fogarty et al. 2012)) between 2130 and 2300 hrs (Eastern Daylight

60 Time, representing ~1.5 to 3 h after sunset) during Augusts of 2007 to 2014. These observations

61 were conducted at four sites in the upper Florida Keys National Marine Sanctuary, spanning a

3

62 distance of approximately 18 km. Genotypes of all individual *A. palmata* colonies within study

63 plots at three of the sites were previously determined and mapped from fixed landmark stakes

64 (Williams et al. 2014). Observation effort was concentrated in the two reef sites with multiple

65 genets present (Elbow and Sand Island reefs) though severe weather sometimes prevented

66 planned observations. More opportunistic observations were collected at sites with a single,

67 abundant genet (Horseshoe Reef, Baums et al. 2005; Molasses Plot 3, Williams et al. 2014) when

68 additional boats and personnel were available. The area of observational coverage at each site

69 was ~ 200–500 m^2, depending on the extent of *A. palmata* occurrence. Observations were made

70 by divers patrolling a circuit among colonies within the observational area to examine polyps for

71 'setting' (the appearance of pink gamete bundles in polyp mouths) and subsequent release of

72 gamete bundles. The *A. palmata* stands at Elbow, Sand Island, and Molasses reefs are typical of

73 remnant fore-reef stands in the area ranging in depth from 3.5 to 4.5 m, whereas the monotypic

74 stand at Horseshoe Reef is an atypically robust thicket occupying a nearshore patch reef (~ 3 m

75 deep).

76   At sites with only a single genet, divers simply observed and recorded substantial (i.e.,

77 >50% of ramets spawning over a substantial portion of their live tissue) or minor (i.e., small

78 patches of tissue releasing few bundles on only one or few ramets) spawning at that site. At

79 diverse sites, genets were identified by arbitrary color names (e.g., Elbow Pink, Sand Island

80 Blue). Divers used underwater maps and color-coded buoys to identify the genet of individual

81 colonies when spawning was observed. Observers at these sites were all very familiar with the

82 layout of colonies and genotypes at these permanent monitoring sites (Williams et al. 2008;

83 Williams and Miller 2012). Observations were only recorded by night and genet; observations at

84 the resolution of minute and ramets within genets were not consistently recorded as divers'

85 attention was diverted to collecting bundles for other studies once spawning began. Bundle

86 release from an individual colony in this species is protracted, generally spanning 15–20 min.

87 A Kruskal–Wallis (nonparametric) ANOVA was used to test the hypothesis that the

88 timing of spawning (i.e., median of any nights within the 2–6 AFM window when any degree of

89 spawning was observed) differed significantly among genets. To further explore what factors

90 best predicted spawning success, an ordered logistic regression model otherwise known as a

91 cumulative link mixed model was developed using the *ordinal* package in R (Christensen 2015).

92 The degree of observed spawning was coded as an ordered response with no spawning < minor <

93 substantial spawning. Year was included as a random effect and the two predictive variables,

94 night after full moon (night AFM) and genet, were included individually and then together to test

95 which model best predicted spawning. Estimation of the predictive variables was via maximum

96 likelihood and the mixed models were fitted with the Laplace approximation (Agresti 2002).

97 Likelihood ratio tests were used to select the best model given the difference in the log likelihood

98 score and the degrees of freedom of the models. P-values were calculated based on the chi-

99 squared distribution. Subsequently, this best fit model was used to calculate the maximum

100 likelihood estimate and standard error of the ordered log odds of each individual genet spawning

101 compared to the genet with the least observed spawning (Elbow pink). The Z-score was used to

102 determine significance in these pairwise comparisons, with p-values less than 0.05 indicating

103 genets with significantly greater odds of spawning on any given night. The exponent of the log

104 odds estimates for individual genets was calculated to obtain the proportional odds ratios for

105 easier interpretation.

106 Temperature loggers (HOBO Pendant) were maintained at Elbow and Molasses reefs

107 over most of the eight years of the study, and at Sand Island since fall 2009, recording every 30

108 min. These data were converted to daily averages and the mean of the three sites was graphed to

109 visualize inter-annual variation in temperature regime between years.

110

Results

112   During three of the eight August spawning windows observed (2012, 2010, and 2007), no

113 spawning by *A. palmata* was observed, and in a fourth year (2011), substantial spawning by only

114 two isolated genets was observed (Fig 1a; hereafter referred to as 'poor' spawning year).

115 Observation during the September window only occurred in 2007, in which a single genet

116 (Elbow gray) was observed to spawn. Observations were greatly curtailed in 2008 due to the

117 occurrence of Tropical Storm Fay so spawning performance during this year is uncertain. Prolific

118 spawning was observed in 2009, 2013 and 2014 with substantial spawning by many genets

119 including co-occurring genets within sites (Fig 1a; hereafter referred to as 'good' spawning

120 years) at a consistent hour (bundles released between ~ 2215 and 2240 hrs EDT). During these

121 'good' years, genets observed to spawn often did not spawn on the same night(s) AFM. However

122 genets showed consistent patterns, spawning with highest probability either on early nights

123 within the window (e.g., Horseshoe), on later nights within the window (e.g., Elbow green), or

124 with little preference (e.g., Elbow orange; Fig 2). A one-way Kruskal–Wallis ANOVA

125 confirmed significant variation among genets in the median night AFM of observed spawning (p

126 = 0.03) though there was inadequate power to distinguish significant post-hoc pairwise

127 differences among individuals.

128   Based on single-factor ordered logistic regression models, night AFM and genet did not

129 significantly differ in their ability to predict spawning. However, a model including both factors

130 (Electronic Supplementary Material Table S1) showed significantly improved performance and

6

131 displayed the lowest AIC score (Table 1). Additionally, three genets had significantly greater

132 odds of spawning on any given night relative to the least-spawning genet (Elbow pink) in

133 pairwise tests (Fig. 2). The genet with the greatest odds of spawning (46.33 greater than Elbow

134 pink) was the single genet at Horseshoe Reef. The genet at Molasses Reef and the orange genet

135 at Elbow Reef also displayed significantly greater relative odds of spawning (14.48 and 8.24,

136 respectively).

137 The temperature record, averaged among Elbow, Sand Island, and Molasses reefs during

138 the period of observation is given in Figure 1b. This temperature record shows no obvious

139 pattern of consistency among nor contrast between 'good' and 'poor' spawning years (Fig. 1).

140 For example, good years included ones with both relatively cold (2009) and relatively warm

141 prior winters while poor years also included cold (2010, 2011) and relatively warm (2007, 2012)

142 prior winters. Similarly, good years appear to have both relatively steep (2009) and less steep or

143 staged (2013) rates of warming during the first half of the year as do poor years (2010 vs 2012).

144

Discussion

146 Even during 'good' spawning years when multiple co-occurring genets spawned,

147 substantial spawning effort was observed on nights and at sites with presumably little potential

148 for fertilization success. Indeed, the model results suggest that the three individual genets with

149 the greatest spawning odds (Horseshoe, Elbow orange, and Molasses; significantly higher odds

150 than the 'base genet' of Elbow pink) each occupy a different site. For example, the monotypic

151 population at Horseshoe Reef is extensive (i.e., > 100 ramets; occupying >1500 m^2 of reef area),

152 comprised of colonies with little partial mortality which spawn profusely (MWM, pers. obs.).

153 However, it was observed to consistently spawn on earlier nights within the window (nights 2–4

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154 AFM; Fig. 2) than the more diverse sites (Elbow and Sand Island, predominantly nights 4–6

155 AFM; Fig. 2), or, in the case of 2011, when none of the diverse sites were observed to spawn at

156 all. In 2011, the Horseshoe genet did spawn the same night as the Molasses genet on night 4

157 AFM, but these sites are over 16 km apart, yielding extremely low expectation that these

158 gametes might mix at adequate concentrations within a few hours to effect fertilization.

159 Similarly, the orange genet at Elbow is also extensive (>30 ramets), displays little partial

160 mortality, and spawns profusely, but exerts a substantial portion of its spawning effort on nights

161 prior to most of its neighboring genets (e.g., 2009 and 2014, Fig 1a).

162   In the observed population, nights 5 and 6 AFM have the greatest genotypic diversity of

163 spawning colonies, implying greater fertilization potential (Levitan et al. 2004; Baums et al.

164 2013; Iwao et al. 2014). The degree of consistent genet-specific preference for spawning on early

165 (Horseshoe) versus later (Elbow white, Elbow green, and Elbow pink) nights within the observed

166 window seems in contrast to the report of no preference by individual *Orbicella franksi* for

167 spawning night within their smaller window (i.e., night 5 versus night 6 AFM; Levitan et al.

168 2011). Rather, genets of *O. franksi* have high hourly synchrony of spawning with proximity

169 among ramets as a secondary correlate of spawn time.

170   There are at least two possible explanations for the lack of observed spawning in certain

171 years: 1) corals may have spawned at a time outside our observations (a different month or lunar

172 nights outside the 2–6 AFM window); or 2) corals may not have spawned at all due to some

173 undescribed natural cycle or overarching stress that precluded physiological investment in

174 spawning (Szmant and Gassman 1990; Lirman 2000; Levitan et al. 2014). Summer 2011 is

175 recognized as a summer with warm temperature bleaching stress in the Florida Keys (Manzello

176 et al. 2015). Daily average temperatures exceeded 31 °C over ten days at the monitored

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177 spawning sites, with mild bleaching observed in *A. palmata* (DEW, pers. obs.). Thus, some

178 degree of warm temperature stress in summer 2011 may correspond to lack of observed

179 spawning in 2012, as shown in *O. faveolata* (Levitan et al. 2014). There was also a severe cold

180 temperature stress event that especially affected nearshore Florida Keys reefs in early 2010

181 (Lirman et al. 2011). However, this event did not appear to impact the *A. palmata* populations at

182 the monitored spawning sites which are restricted to offshore habitats (Fig. 1b this study;

183 Williams and Miller 2012).

184   It is perhaps more plausible that timing of spawning was simply displaced to months or

185 nights beyond our observations during the 'most likely' window. Temperature is understood to

186 be an important determinant of reproductive cycles in corals and thus changing temperature

187 regimes with ocean warming have the clear potential to disrupt coral spawning phenology

188 (discussed in Baird et al. 2009). Seasonal temperatures in the Florida Keys have already shown

189 significant increase over historical baseline (Kuffner et al. 2015; Manzello 2015). However, the

190 temperature record for three of the observed reefs over these eight years does not show obvious

191 consistency among 'good' nor 'bad' spawning years (Fig. 1). There are some isolated reports of

192 observed *A. palmata* spawning in months adjacent to our lack of observed spawning in August

193 (e.g., July 2011 at Looe Key, D. Vaughn pers. comm.; Sept 2007; Fig. 1a) but did not appear to

194 rise to the level of a full spawning event.

195   The rarity of spawning events in the observed population where multiple genets of *A.*

196 *palmata* spawn in proximity on the same night indicate that larval production potential is even

197 lower than the general characteristics of the population (e.g., colony and genotypic density;

198 (Williams et al. 2014)) at these sites would suggest. It is anticipated that warming-induced

199 phenological shifts (Baird et al. 2009), increased barriers to fertilization with ocean acidification

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200   (Albright et al. 2010; Albright and Mason 2013), and potentially other environmental stressors

201   will only worsen processes of depensation in this population in the future. This prospect

202   underscores the pertinence of proactive strategies to enhance both larval production and

203   successful recruitment of larvae as are now called for in the United States' *Acropora* spp.

204   Recovery Plan (NMFS 2015) including potential transplants to increase density of synchronously

205   spawning genets within reef patches.

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Acknowledgements

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References

219   Agresti A (2002) Categorical data analysis, second edition. John Wiley & Sons, Hoboken, NJ,
220   USA
221   Albright R, Mason B (2013) Projected near-future levels of temperature and pCO_2 reduce coral
222   fertilization success. PLoS One 8:e56468
223   Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment
224   success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci U S A
225   107:20400–20404

226 Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the
227   reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551–571
228 Baums IB, Hughes CR, Hellberg M (2005) Mendelian microsatellite loci for the Caribbean hard
229   coral *Acropora palmata*. Mar Ecol Prog Ser 288:115–127
230 Baums IB, Devlin-Durante MK, Polato NR, Xu D, Giri S, Altman NS, Ruiz D, Parkinson JE,
231   Boulay JN (2013) Genotypic variation influences reproductive success and thermal stress
232   tolerance in the reef building coral, *Acropora palmata*. Coral Reefs 32:703–717
233 Christensen RHB (2015) Ordinal — regression models for ordinal data. R package version
234   20151-21 http://wwwcranr-projectorg/package=ordinal/
235 Fogarty ND, Vollmer SV, Levitan DR (2012) Weak prezygotic isolating mechanisms in
236   threatened Caribbean *Acropora* corals. PLoS One 7:e30486
237 Fukami H, Omori M, Shimoike K, Hayashibara T, Hatta M (2003) Ecological and genetic
238   aspects of reproductive isolation by different spawning times in *Acropora* corals. Mar
239   Biol 142:679–684
240 Iwao K, Wada N, Ohdera A, Omori M (2014) How many donor colonies should be cross-
241   fertilized for nursery farming of sexually propagated corals? Natural Resources 5:521–
242   526
243 Kuffner IB, Lidz BH, Hudson JH, Anderson JS (2015) A century of ocean warming on Florida
244   Keys coral reefs: historic in situ observations. Estuaries and Coasts 38:1085–1096
245 Levitan DR, Boudreau W, Jara J, Knowlton N (2014) Long-term reduced spawning in *Orbicella*
246   coral species due to temperature stress. Mar Ecol Prog Ser 515:1–10
247 Levitan DR, Fogarty ND, Jara J, Lotterhos KE, Knowlton N (2011) Genetic, spatial, and
248   temporal components of precise spawning synchrony in reef building corals of the
249   *Montastraea annularis* species complex. Evolution 65:1254–1270
250 Levitan DR, Fukami H, Jara J, Kline D, McGovern TM, McGhee KE, Swanson CA, Knowlton N
251   (2004) Mechanisms of reproductive isolation among sympatric broadcast-spawning
252   corals of the *Montastraea annularis* species complex. Evolution 58:308–323
253 Lirman D (2000) Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth,
254   survivorship, and reproduction of colonies and fragments. J Exp Mar Bio Ecol 251:41–57
255 Lirman D, Schopmeyer S, Manzello D, Gramer LJ, Precht WF, Muller-Karger F, Banks K,
256   Barnes B, Bartels E, Bourque A, Byrne J, Donahue S, Duquesnel J, Fisher L, Gilliam D,
257   Hendee J, Johnson M, Maxwell K, McDevitt E, Monty J, Rueda D, Ruzicka R, Thanner S
258   (2011) Severe 2010 cold-water event caused unprecedented mortality to corals of the
259   Florida reef tract and reversed previous survivorship patterns. PLoS One 6:e23047
260 Manzello DP (2015) Rapid recent warming of coral reefs in the Florida Keys. Sci Rep 5:16762
261 Manzello DP, Enochs IC, Kolodziej G, Carlton R (2015) Recent decade of growth and
262   calcification of *Orbicella faveolata* in the Florida Keys: an inshore–offshore comparison.
263   Mar Ecol Prog Ser 521:81–89
264 NMFS (National Marine Fisheries Service) (2015) Recovery plan for elkhorn (*Acropora*
265   *palmata*) and staghorn (*A.cervciornis*) corals. Prepared by the Acropora Recovery Team
266   for the National Marine Fisheries Service, Silver Spring, Maryland
267 Oliver J, Babcock R (1992) Aspects of the fertilization ecology of broadcast spawning corals:
268   sperm dilution effects and in situ measurements of fertilization. Biol Bull 183:409–417
269 Szmant A, Gassman N (1990) The effects of prolonged "bleaching" on the tissue biomass and
270   reproduction of the reef coral *Montastraea annularis*. Coral Reefs 8:217–224

271 Williams DE, Miller MW (2012) Attributing mortality among drivers of population decline in
272   *Acropora palmata* in the Florida Keys (USA). Coral Reefs 31:369–382
273 Williams DE, Miller MW, Kramer KL (2008) Recruitment failure in Florida Keys *Acropora*
274   *palmata*, a threatened Caribbean coral. Coral Reefs 27:697–705
275 Williams DE, Miller M, Baums I (2014) Cryptic changes in the genetic structure of a highly
276   clonal coral population and the relationship with ecological performance. Coral Reefs
277   33:595–606
278 Wilson J, Harrison P (2003) Spawning patterns of scleractinian corals at the Solitary Islands—a
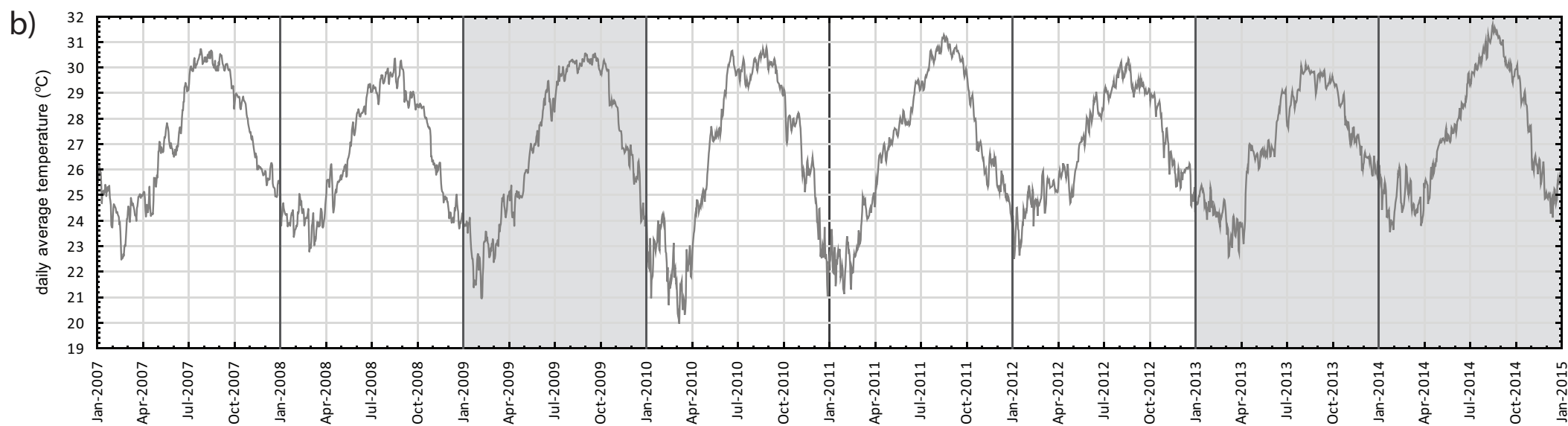279   high latitude coral community in eastern Australia. Mar Ecol Prog Ser 260:115–123
280

281 **Figure captions**

282 **Fig. 1 a**) Spawning observations for twelve genets (depicted as rows) of *Acropora palmata*

283 across four reefs over eight years in the upper Florida Keys. *AFM* = after full moon. *Solid fill*

284 indicates significant spawning while *stippled fill* indicates minor spawning (see text for

285 characterization). *X* indicates no spawning observed. *Empty boxes* indicate that no observations

286 were conducted and *hatching* appears in a row to indicate the time when all colonies of that

287 genet within the observed reef area had died. *Asterisk* acknowledges that night 2 AFM for the

288 Sept 2007 lunar month was actually 31 August. **b**) Daily mean temperatures (based on in situ

289 HOBO Pendant loggers) averaged over three of the observed spawning sites (Elbow, Sand

290 Island, Molasses reefs) over the eight year spawning record. *Shading* highlights 'good' spawning

291 years, when multiple co-occurring genets were observed to spawn

292 **Fig. 2** Frequency of spawning (number of nights observed) by individual *A. palmata* genets over

293 the five-night expected spawning window pooled over eight years. Genets often spawn on more

294 than one consecutive night. *AFM* = after full moon. P-values and proportional odds ratios (OR)

295 depicted on each panel indicate significance of increased overall odds of each genet spawning on

296 any given night (compared with the least-spawning Elbow pink genet) derived from the ordinal

297 logistic regression model with night AFM and genet as predictive factors

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a)

		2007							2008					2009					2010					2011					2012					2013					2014				
		Aug				Sep			Aug					Aug					Aug					Aug					Aug					Aug					Aug				
Night AFM		2	3	4	5	3	4	5	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
Date		1	2	3	4	31*	1	2	18	19	20	21	22	7	8	9	10	11	26	27	28	29	30	15	16	17	18	19	3	4	5	6	7	22	23	24	25	26	12	13	14	15	16

Figure continues as a presence/absence matrix (rows: **Elbow** — Orange, Yellow, Gray, Blue, Green, Pink, White; **Sand Island** — Orange, Blue, Green; Molasses; Horseshoe) across the dates above, with "Tropical Storm Fay" labelled over the 2008 columns.

b)

daily average temperature (°C) plotted from Jan-2007 to Jan-2015.
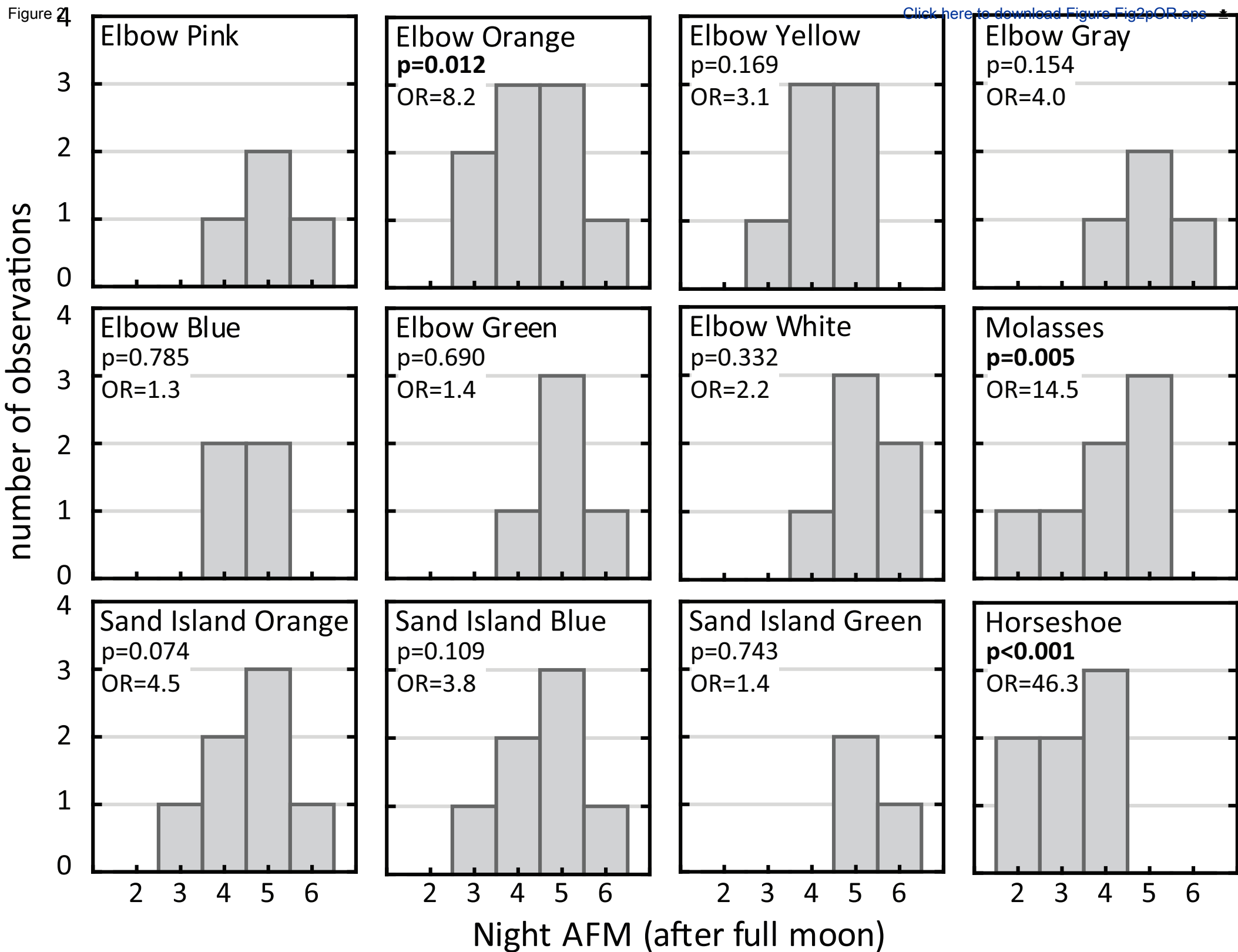
Figure 2

Table 1

Table 1: Likelihood Ratio tests comparing ordered logistic regression models. All models include Year as a random variable. The likelihood ratio test compares the two models identified in the Test column and generates a *p*-value based on the difference in the -2 log likelihood statistic (Lik Ratio stat.) and difference in the degrees of freedom of the model residuals (Diff. in df) using the Chi-squared distribution. AIC indicates the Akaike information criterion for which lower values indicate better models.

Model	Predictive Variable	Residual df	AIC	-2 log Lik	Test	Diff. in df	Lik Ratio stat.	*p*-value (Chi-sq)
A	Night AFM	321	321.28	313.28				
B	Genet	311	339.08	311.08	A vs. B	10	2.20	0.99
C	Night AFM + Genet	310	313.10	283.10	B vs. C	1	27.98	1.22 e-07