Pair bond endurance promotes cooperative food defense and inhibits conflict in coral reef butterflyfish

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Pair bonding is generally linked to monogamous mating systems, where the reproductive benefits of extended mate guarding and/or of bi-parental care are considered key adaptive functions. However, in some species, including coral reef butterflyfishes (f. Chaetodontidae), pair bonding occurs in sexually immature and homosexual partners, and in the absence of parental care, suggesting there must be non-reproductive adaptive benefits of pair bonding. Here, we examined whether pair bonding butterflyfishes cooperate in defense of food, conferring direct benefits to one or both partners. We found that pairs of Chaetodon lunulatus and C. baronessa use contrasting cooperative strategies. In C. lunulatus, both partners mutually defend their territory, while in C. baronessa, males prioritize territory defense; conferring improvements in feeding and energy reserves in both sexes relative to solitary counterparts. We further demonstrate that partner fidelity contributes to this function by showing that re-pairing invokes intra-pair conflict and inhibits cooperatively-derived feeding benefits, and that partner endurance is required for these costs to abate. Overall, our results suggest that in butterflyfishes, pair bonding enhances cooperative defense of prey resources, ultimately benefiting both partners by improving food resource acquisition and energy reserves.
assistance. Although the role of assisted resource defense (ARD) in promoting pair bonding has received less research attention than that of mate-guarding or bi-parental care, in situ observations and explicit tests of these predictions have supported the ARDH for pair bonding across a wide range of taxa (Supplementary Table S1) (but see25,26).

Butterflyfishes of the genus Chaetodon are ideal model taxa for testing the ARDH for pair bonding. Among the ~91 species within the genus, at least 59 reportedly pair bond (data sourced from 12,27–30. Heterosexual pairs of at least some species (mainly, Chaetodon lunulatus) are monogamous12,33. However, same-sexed12,13,34, reproductively immature16,34, and reproductively inactive12,35 pairing also occurs. Moreover, butterflyfishes do not provide parental care12,31,32. At least 24 Chaetodon species feed predominantly (≥80%), if not exclusively, on a diet that is temporally and spatially stable, and therefore economically defendable (i.e., coral)33,35,36,37. There is, however, considerable variation in the level of dietary specialization among corallivorous butterflyfishes that is related to interspecific dominance over feeding sites, such that obligate corallivores dominate territorial disputes over feeding generalists40. Pairing in corallivorous butterflyfishes is suggested to arise from the need for assisted defense of coral prey against con- and heterospecifics in order to better invest in feeding and energy reserves32,33,41. Although pair bonding butterflyfishes are presumed to have very high levels of partner fidelity (up to 7 yrs) (Supplementary Table S2), the ecological basis of pair bond fidelity among these organisms remains unknown.

The overall aim of this study was to test whether pair bonding in two species of common coral-feeding butterflyfishes (C. lunulatus and C. baronessa) may be attributed to benefits of assisted defense of dietary resources, and whether pair bond endurance enhances the effectiveness of assisted resource defense. Specifically, we aimed to test ARDH predictions that either: i) males primarily defend a feeding territory, and females benefit from male assistance by improved investment in feeding and energy reserves, or ii) both partners mutually defend their feeding territory and benefit from each other’s assistance by improved investment in feeding and energy reserves. If so, then finally, we tested the prediction that iii) pair bond endurance reduces intra-pair conflict and/or promotes assisted territory defense and/or energy reserves.

Methods
Study location and model species. This study was conducted on snorkel at adjacent sheltered reefs of Lizard Island, located on the northern Great Barrier Reef, Australia (14°40’S, 145°27’E). To minimize effects of reproductive activity, sampling was conducted January–March (2014) at haphazard times between 0830–1730 h, which are times outside of peak reproductive activity of coral reef fishes at the study location. Sampling was conducted on the two most locally abundant coral-feeding and pair bonding butterflyfishes, C. lunulatus and C. baronessa (Fig. 1). Only individuals that were within 80% of the asymptotic size for the species (C. lunulatus: >64 mm standard length (SL); C. baronessa: >61 mm SL), and therefore likely to be reproductively mature42 were considered. Both species are territorial40 and are predominantly found in long-term, heterosexual partnerships13,34. This study was conducted using Great Barrier Reef Marine Park Authority permits: G10/33239.1, G13/35909.1, G14/37213.1; James Cook University General Fisheries permit: 170251. It was approved by James Cook University Animal Ethics committee (approval # A1874), and performed in accordance with relevant guidelines and regulations.

Coordination and competitor aggression between male and female partners. To test whether pairs displayed either male-prioritized or mutual territory defence, we conducted in situ observations on naturally occurring paired and solitary individuals. Fishes were haphazardly encountered, approached from 2–4 m, and given 3-min to acclimate to observer presence. Following the acclimation period, social system was estimated during a 5-min observation. Pair bonded individuals were identified as displaying coordinated swimming

Figure 1. Chaetodon lunulatus (a) and C. baronessa (b) as model species of pair bonding butterflyfishes used in the current study. At the study location, Lizard Island (GBR), these species are territorial coral feeding specialists that display enduring pair bonds. Pictures are of focal pairs used in this study, taken by J. P. N.
exclusively with another conspecific, whereas solitary individuals were identified as displaying no coordinated swimming with another conspecific. For pair bonds, each individual was then identified using unique body markings (as per32) and assigned an identity number used for ongoing behavioral observations and sexing. Following, levels of coordination and competitor aggression were measured during a further 6-min observation. Level of coordination was measured in pair bonded and solitary individuals, and determined by recording the presence or absence of coordinated swimming every 10-sec. Coordination, defined as the synchronisation of individuals’ movements in space and time45, was considered as the focal fish being positioned within a 2-m distance from another conspecific whilst being faced within a 315-45° angle relative to the faced position of the other conspecific (designated as 0°) (Fig. 2). Coordination was visually estimated after practicing accuracy on dummy fishes prior to the study. Level of aggression toward competitors was measured in male and female partners of pairs, and determined by quantifying the total number of aggressive acts (i.e., staring, chasing, fleeing, encircling, and head down, tail-up displays) expressed (see 46 for detailed descriptions). Only aggression towards other butterflyfishes was measured, because for most butterflyfish, territorial competition is intra-familial47. After each observation, both partners of pairs were collected by spearing through the dorsal musculature and sacrificed in an ice slurry for sex determination. A one-way ANOVA was used to compare the level of pair coordination between C. lunulatus and C. baronessa. Coordination data was square-root transformed prior to analysis to improve normality of residual variance. For each species, a paired t-test was used to compare rate of aggressive acts between males and females within pairs.

Paired vs. solitary individuals: Competitor aggression and feeding bites. To test whether one or both sexes benefit from pairing through reduced competitor aggression or increased feeding rates, we measured and compared these variables between naturally occurring paired and solitary individuals of both sexes. Individuals were considered pair bonded and solitary using the criteria previously described. After establishing their social status and undergoing 3-min acclimation to observer presence, focal individuals underwent a single 6-min observation to record: i) total feeding bite rate, determined by the number of bites taken on any coral ii) total feeding bites on preferred coral types (for C. baronessa only, since this species is a particularly specialized coral feeder, favoring Acropora hyacinthus, A. florida, and Pocillopora damicornis48,49), and iii) total rates of aggression toward neighboring butterflyfish. Rates of aggression may be affected by the local abundance of competitors (independent of levels of aggression exhibited by focal individuals), which was higher in paired than solitary fish. Therefore, in order to account for this potential confound, the number of aggressive acts recorded during replicate observations was standardized to per butterflyfish present within the immediate vicinity (3 m) of the fish’s feeding territory. Immediately following, butterflyfishes were collected by spearing through the dorsal musculature and sacrificed in an ice slurry for sex determination and energy reserves analysis. For each sex of each species, rates of aggression were compared between social conditions using non-parametric Mann-Whitney U tests, due to non-normal distribution of residual variance. For each species, feeding bite rate on total coral was compared between social conditions using a factorial ANOVA (with sex and social condition as fixed factors). For each sex of C. baronessa, a non-parametric Mann-Whitney U test was used to compare feeding bite rate on preferred coral between social conditions, due to non-normal distribution of residual variance.

Figure 2. Coordinated swimming examined in pair bonded and solitary butterflyfish. Coordinated swimming by focal fish (black) was defined as being positioned within a 2-m distance from another conspecific (grey) whilst being faced within a 315-45° angle relative to the faced position of another conspecific (designated as 0°).
Enduring vs. new pairs: Intra-pair relations, and per capita competitor aggression and feeding bites.

We used a partner removal-replacement experiment to examine whether pair bond endurance reduces territory defense or increases feeding of paired individuals by promoting cooperative territory defense and/or reducing intra-pair conflict. Both partners of naturally occurring pair bonds of C. lunulatus (n = 9) and C. baronessa (n = 10) were identified and monitored through time using unique body markings (as per 43), which were photographed and printed on water-proof paper to assist observers (see Supplementary Fig. S2 for example photographs). Pairs were assumed to have been enduring, based on previous research showing a high level of partner endurance in these species at the study location44. Prior to experimentation, one individual from each pair was haphazardly chosen as the focal individual for the experiment. To identify the focal individual and its partners throughout the experiment, a photograph of both lateral sides of their body was taken, from which a unique body markings were recognized and used45. Behavioral expression of the focal individual while with its original partner was measured throughout an 8-min observation, for 5 consecutive days. Prior to each observation, the focal individual and its partner were allowed to acclimate to observer presence for 3-mins (as described above). During each observation, i) time spent coordinated swimming with partner, ii) aggression towards partner, iii) aggression per competitor, and iv) feeding bites of the focal individual were recorded using the methods previously described. Immediately following observations conducted over 5 consecutive days, the partner of the focal individual was removed via spearing and sacrificed in an ice slurry for sex determination and energy reserve analysis. All focal individuals had re-paired with a new partner within 18 hours of partner removal, as determined by identification methods previously described. We then conducted the same behavioral observations for a further 7 (C. lunulatus) or 9 (C. baronessa) consecutive days. After experimentation, the focal individual and its new partner were collected by spearing through the dorsal musculature and sacrificed in an ice slurry to determine the sex of both individuals and energy reserve of the focal individual’s new partner. Temporal changes in time spent coordinated swimming with partner, aggression towards partner, aggression per competitor, and feeding bites were analyzed using multivariate analysis of variance (MANOVA) followed by univariate Tukey’s post hoc tests to identify between-group differences, with results displayed using canonical discriminant analysis (CDA)50,51.

Solitary vs. newly paired vs. enduringly paired individuals: Differences in liver hepatocyte vacuolation.

To assess changes in energy reserves in association with pairing and partner endurance, we compared liver hepatocyte vacuole density between individuals who (i) naturally occurred in solitude (from in situ observation study), (ii) were in new pair bonds (C. lunulatus: 7 day old partnerships; C. baronessa: 9 day old partnerships) and (iii) were in naturally occurring enduring pair bonds (latter two conditions were acquired from individuals from partner removal experiment). Whole livers were dissected and fixed in 4% phosphate-buffered formalin (PBF). Fixed liver tissues were then dehydrated in a graded ethanol series and embedded in paraffin wax blocks. Tissues were sectioned at 5 µm, mounted onto glass slides, and stained using Mayer's hematoxylin and eosin to emphasize hepatocyte vacuoles. Hepatocyte vacuole density was quantified using a Weibel eyepiece to record the proportion of points (out of 121) that intersected with hepatocyte vacuoles when viewed at X 40 magnification. Three estimates of hepatocyte vacuolation were taken for each of 3 cross sections, totaling 9 replicate estimates per fish liver, following53. In both species, differences in the percentage of liver hepatocyte vacuolation between solitary, newly paired, and enduringly paired fish were analyzed using a non-parametric Kruskal-Wallis one-way ANOVA53, due to non-normality in residual variance. Variation in hepatocyte vacuolation could not be analyzed for each sex separately, due to small sample sizes. Tukey and Kramer (Nemenyi) post hoc tests were used to identify differences between social condition means.

Sex determination.

The sex of focal fish was determined histologically. Gonads were removed and fixed in formaldehyde-acetic acid-calcium chloride (FACC) for at least 1 week. Thereafter, gonads were dehydrated in a graded alcohol series, cleared in xylene, embedded in paraffin, sectioned transversely (7 µm thick), and stained with hematoxylin and eosin. Sections were examined under a compound microscope (400 × magnification) for the presence of sperm (male) or oocytes (female)16.

Results

Coordination and competitor aggression between male and female partners. The two study species exhibited contrasting modes of cooperative or assisted territory defense. In both species, solitary individuals displayed no level of coordinated swimming with another conspecific. Pairs of C. lunulatus spent most of their time (56%) swimming with coordination throughout their feeding territory (Fig. 3a; see Supplementary Video S1 for example). When encountering neighboring butterflyfish, both partners displayed equal levels of aggressive acts (t1, = 0.097, p = 0.93; Fig. 3b), suggesting that there is mutual assistance in territory defense. By contrast, C. baronessa partners spent notably less time (10%) swimming with coordination than C. lunulatus (F1,215 = 40.04, p = 0.00) (Fig. 3a). In general, males tended to move over large distances within and along the boundaries of their territory whilst continuously foraging, whereas females tended to restrict movement to areas of their preferred coral (if available) whilst continuously foraging on the outcrop. When territorial disputes occurred, C. baronessa males exerted 48% higher levels of aggression than females (t4 = 3.05, p = 0.04; Fig. 3c), suggesting that in this species, territory defense is male-prioritized.

Paired vs. solitary individuals: Competitor aggression and feeding bites. Across study sites, naturally occurring pairs of both species were common, whereas singletons were rare. A higher abundance of neighboring butterflyfish surrounding paired individual’s territories (in C. lunulatus by ~36%, in C. baronessa by ~75%) was found, suggesting that they had more neighboring competitors than solitary counterparts. For each sex of each species, after standardizing aggression to per competitor present, there was no significant difference in rates of aggression between paired and solitary individuals (C. lunulatus males: z = −0.21, p = 0.93; C. baronessa males:
z = −0.53, p = 0.64; females: z = −0.00, p = 1.00), except for in female *C. lunulatus*, where paired females exerted 71% less aggression per competitor than single females (z = −2.11, p = 0.05; Fig. 4a,c).

In both sexes of both species, paired individuals had higher feeding bite rates than solitary counterparts (*C. lunulatus*: total coral bites: \(F_{1,44} = 28.57, p = 0.00\); *C. baronessa*: total coral bites: \(F_{1,40} = 28.91, p = 0.00\). Preferred coral bites: *C. lunulatus*: total coral bites: \(z = −2.42, p = 0.01\) (males), \(z = −2.88, p = 0.00\) (females), Fig. 4b,d). In *C. lunulatus*, single males took 36.83 ± 8.87 SE bites per 6-min, whereas paired males took 86.21 ± 4.28 SE bites (−57% more); and single females took 37.40 ± 9.41 SE bites per 6-min, whereas paired females took 88.67 ± 8.56 SE bites (approx. 58% more). Consistently, in *C. baronessa*, single females took 46.00 ± 3.21 SE total coral bites per 6-min, among which 1.17 ± 1.17 SE bites were on preferred coral; whereas paired females took 79.67 ± 4.91 SE total coral bites per 6-min, among which 33.33 ± 10.98 SE bites were on preferred coral (−43% more total coral bites, and −96% more preferred coral). Similarly, single males took 37.40 ± 3.93 SE bites per 6-min (1.4 ± 1.16 SE bites on preferred coral), whereas paired males took 73.13 ± 4.86 SE bites per 6-min (32.44 ± 10.54 SE bites on preferred coral), equating to −49% more bites on coral and −96% more bites on preferred corals.

### Enduring vs. new pairs: Intra-pair relations, and per capita competitor aggression and feeding bites.

#### Costs of establishing new partnerships.
Throughout the 5 consecutive days leading up to partner removal, all focal individuals maintained their same partner and territory, and their activity profiles (union of pair swimming, within-pair aggression rate, competitor aggression rate, and feeding bite rate) remained unchanged (*C. lunulatus*: Hotelling’s trace = 0.35, df = 16, p = 0.70; *C. baronessa*: Hotelling’s trace = 0.22, df = 12, p = 0.86; Fig. 4c,d canonical score plots; see Supplementary Fig S1 for behaviour specific profile plots). Within 18 hours of removing their original partner, all focal individuals had kept their same territory, where they had re-paired with a new partner. After re-pairing, the activity profile of fishes dramatically changed (*C. lunulatus*: Hotelling’s trace = 0.23, df = 1, p = 0.00; *C. baronessa*: Hotelling’s trace = 0.29, df = 1, p = 0.00; Fig. 4a,b canonical score plots). This change was mostly attributed to higher within-pair aggression, and to a lesser extent altered coordinated swimming (*C. lunulatus*: reduced coordinated swimming; *C. baronessa*: increased coordinated swimming), higher aggression per neighbouring competitor, and lower total feeding bites than when they were in their enduring partnership (Fig. 5a,b canonical structure plots; see Supplementary Video S2 for example of day 1 of new partnership).

#### Recovery with new partnership endurance.
After re-pairing, focal individuals maintained association with their new partner throughout the remainder of the study (*C. lunulatus*, seven days; *C. baronessa*, nine days), except for one *C. lunulatus* individual, who underwent a second re-pairing 3 days after its original partner was removed. As new pairs endured, focal individuals’ activity profiles significantly changed (*C. lunulatus*: Hotelling’s trace = 0.77, df = 24, p = 0.03; *C. baronessa*: Hotelling’s trace = 1.25, df = 32, p = 0.00; Fig. 5c,d canonical score plots; see Supplementary Fig S1 for behaviour specific profile plots). This change was mostly attributed to a reduction in intra-pair aggression, and to a lesser extent to adjusted coordinated swimming (*C. lunulatus*: increased; *C. baronessa*: decreased), reduced aggression per competitor, and increased feeding bites (Fig. 5c,d canonical structure plots). Within 4 days of forming a new partnership, activity profiles recovered to levels displayed by original pairs.
Energy reserves of solitary vs. newly paired vs. enduringly paired individuals. For both *C. baronessa* and *C. lunulatus*, liver hepatocyte vacuole density varied significantly with social condition (*C. lunulatus*: Kruskal-Wallis = 19.39, df = 2, p = 0.00; *C. baronessa*: Kruskal-Wallis = 10.27, df = 2, p = 0.01). While there was no difference in liver vacuole density between individuals that were in enduring and relatively new (i.e., that persisted for 7–9 days) partnerships, paired individuals had greater hepatocyte vacuolation than solitary counterparts (Fig. 6a,b).

Discussion

In this study, we provide field-based observational evidence for the ARDH for pair bonding in two species of butterflyfishes. We show that pairs of *C. baronessa* and *C. lunulatus* appear to exhibit alternative modes of assisted territory defense (male-prioritized and mutual defense, respectively) that is associated with increased feeding bites relative to solitary counterparts. We furthermore provide the first evidence that this feeding bite advantage translates into significant gains in energy reserves in butterflyfishes. Finally, this is one of the first studies to examine why organisms that exhibit ARD have long-term partnerships, providing experimental evidence that partner endurance plays a critical role by inhibiting conflict and promoting ARD between partners.

It has previously been proposed that, where ARD drives pairing, males nonetheless take-on the greatest burden of defense. Although this is largely consistent with our results for *C. baronessa*, our findings for *C. lunulatus* contribute to a growing body of literature indicating that males and females may contribute equally to resource defense, because both may equally benefit from each other’s assistance (Supplementary Table S1). Partners frequently swam with coordination while foraging throughout their territory. When encountering neighboring butterflyfishes, aggression was generally passive, consistent with some other butterflyfishes. Presumably, the function of pair swimming in butterflyfish pair bonds may be akin to duetting in bird pair bonds, in that it conspicuously advertises territory occupancy, thereby avoiding usurpation attempts by neighbors. Notably, when territorial aggression did occur, it was exerted by both partners mutually. For both sexes, this ostensive co-defense in paired individuals was associated with an improved feeding bite rate (by ~58%), and energy reserves (by 69%, as indicated by hepatocyte vacuolation) relative to solitary counterparts. Consistently, in *C. chrysurus* (=*paucifasciatus*), male-female partners continuously travel closely together throughout their territory, mutually engage in territory defense, and both partners have higher feeding rates than solitary individuals.

In contrast to *C. lunulatus*, territory defense by pairs of *C. baronessa* appeared to be male-prioritized. Partners frequently traveled independently from each other, spending only ~10% of their time swimming with...
coordination. For most time (~90%) males independently patrolled larger areas within and along the boundaries of territories, exerting ~48% more aggression towards neighboring butterflyfishes than females. This seemed to allow females to mainly focus on foraging, notably in a more restricted area within the territory that contained a dominant assemblage of preferred coral (e.g., *A. hyacinthus* or *A. florida*). In association, paired females bit 43% more total coral, and 96% more preferred coral than their solitary counterparts. Moreover, and probably because of increased food bites, liver lipid reserves in paired individuals were higher by ~57% than solitary individuals, though no distinction was made between males versus females. Among species that pair for assisted resource defense purposes, male-prioritized defense is the most commonly reported mode of assistance (Supplementary Table S1), where it has been attributed to supporting female food consumption in birds and butterflyfishes, presumably enabling the male to share his mate's subsequent increased fecundity. This form of sexual division-of-labor is thought to occur when female egg production is especially costly, and disproportionally more male assistance is required for females to build the energy reserves needed for egg production. Egg production in female *C. baronessa* may be particularly energetically costly, thereby favoring male-prioritized over mutual defense. Although some of *C. baronessa*’s preferred diet, *Acropora* corals, provide the best energetic return among coral families (after accounting for feeding efficiency), they nonetheless exhibit higher feeding rates on coral.
tissue per day than *C. lunulatus* and other congeners\textsuperscript{39}, indicating that perhaps they have a relatively low energetic absorption efficiency. To ascertain this possibility, analysis of energetic absorption efficiency (relative to other corallivorous species) would be required. Interestingly, despite pair bonded male *C. baronessa* exerting more territory defense effort than females, pair bonded males also conferred feeding benefits, as they bit 49\% more total, and 96\% more preferred coral than their solitary counterparts. This might suggest that in addition to presumably sharing in female's increased fecundity, pairing males might also directly confer an advantage to food consumption, due to the (albeit relatively little) territorial defense assistance provided by females.

In previous studies of pair bonding butterflyfishes, ARD led to increased feeding rates by reducing the time needed for territory defense, thereby providing more time to invest in feeding\textsuperscript{12,41}. In our study, since pair bonded *C. lunulatus* exhibited mutual ARD, we expected that both males and females would confer reductions in territory defense; and since pair bonded *C. baronessa* exhibited male-prioritized ARD, we expected that males would be burdened with increased territory defense while females would benefit from reductions in this task. This expectation was met for female *C. lunulatus*, as pair bonders exhibiting mutual ARD and increased feeding also conferred a reduction in territory defense relative to solitary, non-assisting counterparts. While the same pattern was found for male *C. lunulatus*; this was highly statistically insignificant. Likewise, in accord with expectation for pair bonded *C. baronessa*, there was a pattern for territory aggression to increase for males and decrease for females relative to solitary counterparts; however, this was also highly insignificant. Importantly, rates of aggression tended to be highly variable for solitary individuals. This, coupled with low sample sizes, limited power to detect potential differences in aggression between paired and solitary individuals. Alternatively, ARD may have enabled pairs to establish territories with greater food availability, thereby providing feeding and energy reserve benefits independently of reduced per capita territory defense. It may also be argued that rather than ARD, increased feeding and energy reserves might be consequent of other function(s) of pairing (e.g., increased predator vigilance)\textsuperscript{36,60}. Finally, it is conceivable that higher food consumption and energy reserves promoted pair bonding. Nevertheless, a causal effect of ARD on improved feeding experimentally shown in other pair bonding butterflyfish species\textsuperscript{12,43} further supports the idea that it also exist in species of the current study. To be certain, however, this should now be confirmed experimentally.

Although species that pair for ARD display long-term partner fidelity, reasons for this are almost wholly unknown\textsuperscript{12,23,33,37,61–70} (Supplementary Table S2). In the current study, we show that within 18 hours of removing their original partner, all remaining fish had kept their same territory, wherein they had re-paired with a new partner. This indicates strong territory fidelity. It moreover suggests that while there is strong pressure to be paired, this is not due to partner/mate scarcity. There are, however, definite benefits of pair bond endurance. Experimentally inducing new partnerships caused an immediate and marked decline in partner relations, although these were relatively short-lived. This decline was mostly attributed to increased intra-pair aggression, primarily from the widower towards the new partner, and to a lesser extent to reduced expression of species-specific modes of ARD, as indicated by decreased pair swimming in *C. lunulatus*, and increased pair swimming in *C. baronessa*. This decline in partner relations appeared to initiate heightened territorial activity with neighbouring butterflyfish pairs. Subsequently, newly paired individuals suffered from having to shift investment from feeding to territory defense. As new partnerships subsequently endured, however, these intra- and inter-pair disruptions abated, and incurred costs to individual territory defence–feeding budgets recovered accordingly. Similarly, it has been shown (in wood louse, *Hemilepistus reaumurii*) that widowed individuals with established territories will initially aggressively resist the elicitation to form new partnerships prior to conceding\textsuperscript{20}. It has also been shown (in barnacle geese, *Branta leucopsis*)\textsuperscript{21,56} that pair bonds of longer duration monopolize higher quality feeding territories, ostensibly through enhanced cooperation, and this is further linked to improvements in life-time reproductive success. Overall, our results suggest that partner fidelity is exhibited in *Chaetodon* butterflyfishes because it plays a critical role in promoting assisted resource defence, and inhibits intra- and inter-pair conflict, ultimately conferring feeding investment gains. Although we found no evidence that this translates into energy reserve gains, this may be consequent of limited sample size and/or sampling after new pairs had already endured for 7–9 days, when they displayed fully-recovered behavioral and energetic profiles. Indeed, in fishes, liver hepatocyte vacuole density has been shown to respond rapidly to changes in feeding (i.e., within 5–8 days)\textsuperscript{71,72}. To ascertain this, energy

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**Figure 6.** Variation in liver hepatocyte vacuole density among individuals in enduring partnerships, new partnerships (7–9-day persistence), and living in solitude. (a) *C. lunulatus*; (b) *C. baronessa*. Data are represented as the mean ± SE. Kruskal–Wallis *p* value is shown in top corners, while groups not sharing the same letter are significantly different [Tukey and Kramer (Nemenyi) post-hoc analysis at *p* < 0.05]. Sample sizes are listed below each treatment group.
reserves should be re-sampled in more individuals and on a shorter time-scale throughout the development of new partnerships. Of course, there may be other reasons for long-term partner fidelity among pair bonding species that exhibit ARD. These might include partners experiencing a delay in the time at which their services are reciprocated. For example, if male assistance is based on increasing female feeding to share her improved fecundity, then males may remain with females across reproductive periods if there is a time-lag between enhanced female feeding and egg production. Partner fidelity may also be attributed to mutual site-attachment to the feeding territory, which may arise if new territories are scarce or competitively costly to acquire.

How and why might partner fidelity promote ARD and inhibit intra-pair conflict in these species? Perhaps partner fidelity improves ARD through partner familiarity. Indeed, it has been shown in fishes that cooperation with specific partners stabilizes over time, because individuals are more cooperative with familiar partners. The mechanism(s) for this may be unique to the species-specific mode of assistance. To work together simultaneously to provide mutual assistance, and as such, familiarity may facilitate learning and accurate prediction of partner behavior (e.g., chosen defense route or routine), thereby fine-tuning pair-wise coordination. For , partners appear to exhibit male-prioritized assistance in exchange for sequentially reciprocated partitioning of services/resources by females (i.e., direct reciprocity), and as such, partner familiarity may allow individuals to learn which “partner control mechanism” is best suited to stabilize cooperation, based on the tendency of partners to reciprocate (or cheat) in the past. Upon new pair formation, partner familiarity (and therefore effective cooperation, and cooperatively derived feeding benefits) takes several days to develop; however, the cost of food sharing is immediately incurred. Hence, until cooperative relations develop, the costs (food sharing) likely outweigh the benefits (maximizing feeding investment) of pairing, causing territory holders to aggressively resist new partner elicitation.

Energy acquisition is fundamental to growth, reproduction, and maintenance for all animals. However, corallivorous butterflyfishes rely almost exclusively on a diet of hard coral, which is a relatively nutrient poor, but abundant resource. Consequently, both sexes are energy maximizers, feeding almost continuously. Foraging is therefore constrained by time spent on other activities, including territory defense. As such, attributes that alleviate time constraints on foraging are likely to directly benefit individual fitness. This study suggests that for and , pair bonded partners use territorial defense assistance to increase their feeding of coral food and energy reserves. We further show that partner fidelity plays a critical role in this function by inhibiting intra- and inter-pair conflict and promoting territorial defense assistance between partners, providing an ecological advantage to pair formation and fidelity in these species. Whether this translates into an adaptive advantage should now be addressed by undertaking long-term monitoring studies to discern whether long-term pair bonding also confers improved survivorship and/or life-time fitness.

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Acknowledgements
We thank Manuela Giammusso, Patrick Smallhorn-West, Andrés Jácome, and staff at Lizard Island Research Station for field assistance. We acknowledge the fishes that were used in the current study. This research was supported by an ARC research grant to M.S.P and S.P.W.

Author Contributions
J.P.N., S.P.W. and M.S.P. designed the study; J.P.N., D.J.C. and K.J.N. collected the data; J.P.N., S.P.W. and K.J.N. analyzed the data, J.P.N. wrote the manuscript, and all authors contributed to reviewing and editing the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-24412-0.

Competing Interests: The authors declare no competing interests.

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