



Experimental analysis of monogamy in the Caribbean cleaner goby, *Gobiosoma evelynae*

JEFFERY A. HARDING, GLENN R. ALMANY, LYNNE D. HOUCK & MARK A. HIXON

Department of Zoology, Oregon State University

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To investigate factors promoting monogamy, we studied the reproductive behaviour and ecology of the monogamous Caribbean cleaner goby at two sites near St Croix, U.S. Virgin Islands. We assessed mate fidelity and the amount of time required to acquire a new mate in experimentally widowed males and females. We also measured behavioural responses of pair members and single females to experimentally introduced conspecific intruders of both sexes. Finally, we evaluated the distribution of suitable habitat relative to existing goby territories. We found that pairs often separated due to males abandoning females and moving to a new territory, that most widowed fish soon acquired a new mate, and that there was strong aggression towards large same-sex intruders. We also found an abundance of suitable, unoccupied habitat. We conclude that pairs are maintained by intrasexual aggression that is related to both mate availability and other resources, such as high-quality, food-rich cleaning stations. Combined with observations of frequent pair separation, interterritory male movement, and relatively rapid remating by both sexes, these results suggest a complex mating system that is best classified as serial monogamy.

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Mating systems in vertebrates span a diverse array, from promiscuous liaisons to polygynous harems to life-long monogamy. Monogamy in vertebrates is uncommon (e.g. Emlen & Oring 1977; Wittenberger 1979). Exceptional in this regard are avian species, the majority of which are monogamous (Kendeigh 1952; Lack 1968). The primary explanation for this avian pattern is that parental efforts are required from the male, as well as the female, to fledge young successfully. Thus, both the female and the male typically cannot increase their individual reproductive success by seeking additional mates.

The nature of monogamous mating in avian species may lead to the assumption that monogamy in general is characterized by some form of biparental care. A consideration of monogamous species in other vertebrate groups, however, quickly dispels this notion. In studies of coral reef fish, for example, monogamous pairs most typically are those in which only the male delivers parental care, usually by guarding the eggs (Barlow 1981, 1984). In these species, factors other than a necessity for

biparental care have promoted the development of monogamous relationships. In an attempt to discover patterns that explain the nature of monogamy, the general correlation between a suite of ecological or social conditions and particular mating systems, including monogamy, have been considered in detail (Orrians 1969; Emlen & Oring 1977). In species lacking biparental care, certain key criteria tend to promote reproductive success for individuals engaged in a monogamous relationship. Two of these criteria are: (1) limited availability of an essential resource: for example, if habitat suitable for nest sites or territories is sufficiently patchy, such that a male can control only a single limited area, and each patch contains resources sufficient for only one pair; and (2) dispersed distribution of mates, such that a male cannot monopolize more than one female. For some species, an additional criterion that may favour monogamy occurs when male mobility is limited by having to provide parental care at a fixed site (e.g. guarding eggs in a nest or burrow). Thus, a more general understanding of the ecological conditions that favour monogamy in vertebrates may be promoted by studies of nonavian species.

Field observations of fish mating systems are relatively limited, compared with the literature available for birds and mammals. However, the accessibility of SCUBA within the last three decades has allowed researchers to make observations of fish behaviour in natural habitats.

Correspondence and present address: M. A. Hixon, 3029 Cordley Hall, Department of Zoology, Oregon State University, Corvallis, OR 97331, U.S.A. (email: hixonm@science.oregonstate.edu). J. A. Harding is now at the Department of Ecology & Evolutionary Biology, Long Marine Laboratory, 100 Shaffer Road, University of California, Santa Cruz, CA 95060, U.S.A. G. R. Almany is now at the School of Marine Biology and Aquaculture, James Cook University, Townsville QLD 4811, Australia.

The most common mating system to emerge from these field studies is polygynandry, which is typified by male territorial defence of a nesting site, the attraction of multiple female mates to that site, male-only parental care, and females mating with a different male for subsequent clutches (e.g. Gross & Sargent 1985). In general, no pair bond or long-term interactions occur between a male and female. In this polygynandrous system, mating or nesting sites typically are limited, and successful males exclude rivals from these sites (but see Gross 1996). However, recent studies have revealed monogamous exceptions to this general pattern. In particular, studies of coral reef fish have provided details of nest site availability and site fidelity, in addition to detailed observations of mating behaviour (Clark & Pohle 1992; Kuwamura et al. 1993; Reavis 1997b; Kokita & Nakazono 1998). In general, male-only parental care is typical of smaller species of coral reef fish that deposit their eggs on the substrate (in contrast to larger species, which are typically broadcast spawners and thus have no parental care; Barlow 1981).

Before we further examine mating systems in coral reef fish, a definition of monogamy is warranted. Several reviews have examined the various natures of monogamous relationships (Barlow 1981, 1984, 1986; Wickler & Seibt 1983; Mock & Fujioka 1990). We adapt these considerations for reef fish by defining monogamy as a long-lasting association (at least two complete spawning cycles) between a male and female pair that includes: (1) prolonged close physical proximity between pair members, often characterized by coordinated movements between the male and female; (2) joint occupancy and defence of a territory or nesting site to the exclusion of other reproductive adults; and (3) spawning that occurs primarily (if not exclusively) between pair members, such that the territorial male sires all (or most) of the offspring produced in his territory. We further define the special case of serial monogamy as a sequence of monogamous relationships, each with a different partner.

Monogamy in coral reef fish has evolved in at least 14 families, and biparental care is rare (Robertson et al. 1979; Reese 1991; and see reviews by Barlow 1984, 1986). In many species, monogamy is associated with low adult densities, readily available habitat, and the absence of alternative mating tactics (e.g. no satellite males). These general trends are characteristic of gobiid reef fish. Gobies (family Gobiidae) are a highly speciose group with over 200 genera and 1875 species (Nelson 1994). Most species appear to spawn promiscuously, although individuals may maintain small, contiguous territories (Thresher 1984). In contrast, a relatively small number of gobies have monogamous relationships, and monogamy has evolved multiple times in this group (Barlow 1984). Monogamy has been described for species in seven gobiid genera, including *Valencienna* spp. and *Gobiosoma* spp. (Nelson 1994). In these monogamous pairs, each individual is aggressive towards conspecifics of the same sex, and defends a territory that includes a burrow or crevice where eggs are laid and then guarded by the male. A monogamous relationship has been described in detail for the Indo-Pacific goby *Valencienna strigata* by Reavis

(1997a, b) and Reavis & Barlow (1998). These authors speculated that *V. strigata* populations were limited by predation on adults, resulting in excess available habitat.

In contrast to *V. strigata*, we studied the mating system of the closely related *G. evelynae*, a Caribbean species that does not seem to be limited by predation on adults. Our initial observations supported earlier suggestions that *G. evelynae* mate monogamously. *Gobiosoma evelynae* is a small (<50 mm total length (TL)), site-attached goby that occurs singly or in male-female pairs as adults (Colin 1975). Males guard demersal eggs that are placed inside small holes and crevices by the female (Thresher 1984). Crevices consist of small cracks or holes between or within live coral or dead coral rubble. Crevices are a natural and abundant feature of coral reefs and thus unlikely to be limiting. Given the small size of *G. evelynae*, potential shelter sites are abundant, although individuals do not seek refuge on the approach of either a diver or other fish (personal observation). Unlike *V. strigata*, *G. evelynae* is an obligate cleaner fish that removes ectoparasites from other fish (Randall 1967; Bohlke & Chaplin 1993). Predation on adults is unlikely to limit *G. evelynae* population density because *G. evelynae* is rarely consumed by predators (Darcy et al. 1974).

We conducted a field study to test the hypothesis that *G. evelynae* is indeed monogamous, and to assess the relationship between available nesting habitat and adult abundance at two study sites. Our specific goals were to: (1) characterize male and female reproductive interactions, (2) estimate the availability of nesting sites within the study areas, and (3) interpret the distribution of mated pairs in relation to available nesting habitat.

METHODS

This study was conducted off the southwestern shore of St Thomas, U.S. Virgin Islands, from June to September 1991 and 1992. All work was performed using SCUBA on the shallow (4–12 m) reefs surrounding two small islets, Saba Cay and Flat Cay. We established two fixed rectangular survey grids in 1991, one at each site, and divided them into 5 × 5 m squares using string and metal stakes. The survey grid was 20 × 50 m at Saba Cay and 10 × 40 m at Flat Cay. A larger grid was used at Saba Cay because goby density was over 50% lower relative to Flat Cay in 1991. The survey grid at Flat Cay was enlarged to 20 × 40 m in 1992 to equalize the number of pairs monitored at each site.

We individually marked and measured all adult *G. evelynae* (>20 mm TL) within each survey grid. We captured fish with a dilute quinaldine/ethanol solution and an aquarium net, and marked them in one of two ways: (1) subcutaneous injection of Liquatex brand coloured acrylic latex paint; or (2) nicking the yellow and white lateral stripe posterior to each eye with the tip of a needle. The second method left a small scar, or set of scars, unique to each individual. We measured each fish to the nearest 0.1 mm TL and returned it to its site of capture. The entire procedure was performed underwater and required 2–3 min per individual. After release, we

observed each fish until it had recovered from the anaesthetic (approximately 5 min). There were no immediately observable adverse effects of capture or marking. However, to test for injury or mortality caused by these procedures, we placed 10 newly marked individuals in a weighted glass aquarium within the survey grid and noted their condition after 24 and 48 h; these fish showed no adverse effects of capture and marking. Furthermore, at Flat Cay we monitored 15 marked pairs in 1991 and 52 marked pairs in 1992. Similarly, we monitored eight and six marked pairs at Saba Cay in 1991 and 1992, respectively. Recaptured fish from both sites showed no signs of infection due to marking.

Gobiosoma evelynae are sexually monomorphic, making sex determination *in situ* difficult (Colin 1975). The female of a pair, however, could often be identified by her egg-distended abdomen, which is visible for 2–3 days prior to spawning. In addition, developing eggs were often visible through her semi-transparent abdominal wall. To confirm the sex of individuals, we collected 55 single individuals and 25 pairs during the study and sexed each animal by dissection (Colin 1975; Robertson & Justines 1982). Examination of gonads and urogenital papillae confirmed that pairs always consisted of one male and one female.

During the two summer breeding seasons of this study, we observed *G. evelynae* pairs spawning repeatedly and regularly. Spawning cycle varied from 4 days to more than 16 days, averaging 6–8 days, and there was no apparent synchrony or periodicity of spawning among pairs; on average, 34% of females were gravid during any single census (Harding 1993).

Pair Characteristics and Mate Fidelity

To determine whether pairing was random by size, we measured the total length of each male and female in 116 pairs. These pairs were randomly selected from both sites in both 1991 and 1992. We regressed male size against female size and used a paired *t* test to examine the size relationship between pair members. To assess the fidelity of marked males and females to their partners and territory sites, we censused Flat Cay every other day from 1 August to 14 September 1991 (23 censuses), and Saba Cay every 3–4 days from 29 July to 13 September 1991 (14 censuses). In 1992, we censused Flat Cay every 2–5 days from 15 June to 14 September (35 censuses), and Saba Cay once per week from 2 July to 10 September (11 censuses). For this analysis, we defined a pair as two marked fish that were seen together on two consecutive censuses and for which there were no subsequent absences or sightings of either pair member with another fish. The large size of each survey grid prevented a thorough search of the grid during each census, so we checked known territories by searching an area within 6 m of the original marking site. Marked fish not located within this area were considered 'disappearances' and those found within this area but not with their original mate as 'moved'. We used a binomial test to determine whether fidelity was related to sex (Zar 1984).

Remating

To determine the average time necessary for artificially widowed fish to remate (acquire a new mate), we conducted a mate removal experiment, outside the two survey grids, in which either the male or female was captured and removed from its partner. In 1991, we removed a total of 32 gobies: 14 at Saba Cay and 18 at Flat Cay. All pairs included in the experiment met three criteria: (1) both fish in the pair were marked; (2) the same two marked fish had been together at one location for at least 8 days; and (3) the pair was not the nearest neighbour of any other pair involved in the experiment.

We removed one fish at random from each pair, subject to the constraint that approximately equal numbers of males and females were removed. Fifteen pairs had not been sexed at the time of the experiment; from these, we removed one fish at random and preserved it for sex determination. The sex of the remaining 17 fish was known prior to removal. We translocated removed fish to an area more than 200 m away from the survey grid.

After mate removal, we monitored the location (present on original coral head, moved to new coral head, or absent) and status (paired or unpaired) of the remaining individual. If a new pair formed, we captured and measured the new fish and checked the reproductive status (gravid or not gravid) of the female. To account for effects of local density on the movement and pairing of these newly solitary gobies, we counted all conspecifics larger than 20 mm TL within a 6-m radius of experimentally widowed fish.

Responses of Pair Members to Staged Territorial Intrusions

We observed the responses of males and females in 12 pairs when conspecific intruders were introduced to their territory. Each pair was challenged with four types of intruders: (1) a male smaller than the resident male, (2) a male larger than the resident male, (3) a female smaller than the resident female and (4) a female larger than the resident female. The 24 gobies in the 12 resident pairs were each individually marked prior to the experiment and the identity of the female in each pair was known. Males were ($\bar{X} \pm \text{SD}$) 28.7 ± 3.0 mm TL and females were 28.1 ± 2.0 mm TL. The sex of each intruder was unknown at the time of introduction so it was necessary to introduce more than four gobies to each pair to complete all four treatments. After each trial we recaptured and preserved each intruder for sex determination later that day. Each intruder was used in a trial the same day it was captured. Resident pairs were challenged with no more than two intruders per day and trials were separated by no less than 2 h to prevent progressive effects of introductions on pair behaviour. Intruder introductions were random by size and blind by sex. Each intruder was released on to the coral head as close to the resident pair as possible and we began timing the trial when one of the residents was within one body length of the intruder. We observed the responses of the residents and recorded three distinct types of aggressive behaviour: (1) biting, (2)

threat displays (residents flared their fins and slowly circled the intruder), and (3) chases (rapid pursuits with no contact between resident and intruder). Each trial lasted between 8 and 30 min ($\bar{X} \pm \text{SD} = 15.5 \pm 6$ min). Residents often expelled intruders from the territory through immediate and persistent aggression. If the intruder was expelled, we stopped the trial, recaptured the intruder with a hand net, and immediately returned it to the pair's territory to allow both the male and female of the pair to interact with the intruder at least once. Most intruders were expelled, recaptured and returned to the resident's territory at least once.

We calculated a behavioural rate response for each resident male and female. The behavioural rate equalled the total number of times each behaviour was performed during a trial divided by the total time of the trial.

We compared the mean responses to each of the four intruder types using a Friedman's two-way analysis of variance (ANOVA). The null hypothesis was that the resident's response did not depend on the sex or size of the intruder. If we rejected this hypothesis, we used a nonparametric a posteriori Tukey-type multiple comparison tests to identify specific differences between the resident's responses (Zar 1984).

Responses of Unpaired Females to Staged Territorial Intrusions

We challenged six unpaired females with both a male and a female intruder in separate trials. The order of addition (male–female or female–male) was random. The six male intruders ($\bar{X} \pm \text{SD} = 29.8 \pm 1.7$ mm TL) and six female intruders ($\bar{X} \pm \text{SD} = 30.0 \pm 2.4$ mm TL) were larger than the six unpaired resident females ($\bar{X} \pm \text{SD} = 26.6 \pm 2.4$ mm TL). Unpaired females had been observed with males at other times during the study, suggesting that their unpaired status was probably a temporary condition. During these trials however, no males were present, so aggressive behaviour displayed by unpaired females could not be interpreted as mate guarding. Trials lasted for 4–10 min ($\bar{X} \pm \text{SD} = 7 \pm 2$ min, $N = 12$). After each trial the intruder was recaptured, preserved and sexed by dissection. We used the Wilcoxon two-sample test to compare behavioural responses to male and female intruders (Zar 1984).

Distribution of Paired and Unpaired Gobies in Relation to Suitable Habitat

We related the distribution of territories of paired and unpaired gobies to the distribution of suitable nesting and territory habitat within our two study grids. We defined suitable habitat based on two characteristics of occupied territories. We measured the maximum length and width of 104 pair-occupied coral heads. Each coral head was selected haphazardly from reef areas within, and adjacent to, the survey grid at Flat Cay. We used these measurements to determine the smallest coral head occupied by a pair for the seven most commonly used coral species (*Montastrea annularis*, *M. cavernosa*,

Siderastrea siderea, *Meandrina meandrites*, *Diploria labyrinthiformis*, *D. strigosa* and *Colpophyllia natans*). We produced detailed maps of the locations of all living coral heads in each survey grid and identified each coral to species. For each coral species, we omitted all heads smaller than the smallest head on which a pair of gobies was observed. We also omitted coral species not used by *G. evelynae* (i.e. *Acropora* spp., *Porites* spp., *Dendrogyra cylindrus* and *Millepora* spp.). This resulted in 487 'inhabitable' coral heads at Flat Cay and 595 'inhabitable' coral heads at Saba Cay. We used image-analysis software (Image 1.41, N.I.H.) to compute planar areas and generate X–Y coordinates for these inhabitable coral heads. We analysed their spatial distribution using the Clark & Evans method of nearest-neighbour analysis (Krebs 1989), corrected for lack of a border strip on the three sides of each survey grid not touching sand. Similarly, we mapped the location of all coral heads occupied by single or paired adult *G. evelynae* within each survey grid and used the Clark & Evans method to analyse the spatial distribution of all female-occupied coral heads at both sites in 1991 and 1992.

To determine whether male territories could encompass more than one female's territory, we estimated male territory size based on the location of nest crevices relative to the male's normal resting spot and by observing the distance males chased intruders from their normal resting spot. We then mapped the location of male territories on to the maps of suitable habitat and female territory distribution.

Ethical note

Marking. Capture-and-mark procedures were deemed necessary to accurately identify pair members for the pair fidelity study, an important part of establishing whether this species is monogamous. No alternative methods that would have been less disruptive were available to accomplish our experimental goal.

Relocation. Fish removed from the survey grid were relocated to vacant coral heads of the appropriate size and species (based on detailed observations of the characteristics of numerous occupied coral heads). Based on our observations of frequent natural movement of individuals from and between territories, we had no reason to believe that relocated gobies would experience any difficulty securing a new territory or would experience any detrimental effects of being relocated.

Encounters. Our initial observations of natural encounters between territory holders and intruders revealed a suite of individual behaviours (e.g. circling behaviour and fin-flaring) that took place during encounters. Because these behaviours would have been impossible to duplicate using a model and, we felt, were an important part of the interactions between individuals, we used live subjects for all staged encounters. We conducted the minimum number of trials necessary to provide a statistically meaningful test. Each trial continued until each member of a pair had approached and inspected the intruder at

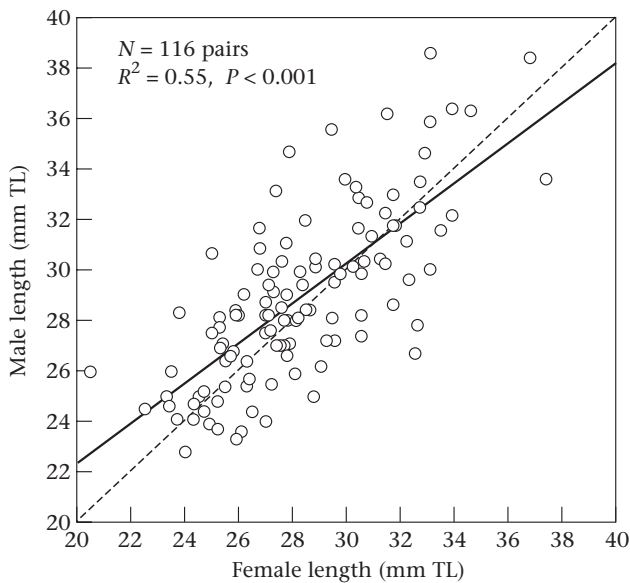


Figure 1. Relationship between length of males and females in 116 *G. evelynae* pairs. Each point represents one pair. The slope of the solid regression line (0.79) differs significantly from zero (see text). Most points fall above the dashed line of equality indicating that, on average, males were larger than their female partners.

least once. Because fish could easily avoid and end encounters by exiting the territory, we never had to intervene during a trial to prevent aggression from reaching a deleterious level. When biting did occur, there was no noticeable injury to any of the test subjects (*G. evelynae* are small and do not have the well-developed dentition that would be required to break the skin or even cause a minor abrasion). We never saw any lesion formation or subsequent infection on subjects, and there were no deaths as the result of any of our trials.

RESULTS

In 1991, we monitored 23 male–female pairs of *G. evelynae* for 48 days. These pairs persisted for an average \pm SD of 26 ± 15 days (range 3–47 days). In 1992, we monitored 58 pairs for 92 days. These pairs persisted for an average of 38 ± 27 days (range 3–92 days). These results may underestimate actual pair persistence because each pair was found together when first marked and/or was still together at the last census (Harding 1993). Not only were pairs persistent, but data from 1992 (see Fig. 4) show that *G. evelynae* typically were found in pairs ($N=36$) versus singly ($N=3$ females).

Pair Characteristics and Mate Fidelity

Because there were no significant between-year or between-site differences, we pooled measurements across sites and years. We found a strong positive relationship between male and female size in pairs of *G. evelynae* (paired t test: $t_{114}=11.7$, $P<0.001$, $r^2=0.55$; Fig. 1). Males were slightly but significantly longer than their female partners when sites and years were pooled ($t_{114}=2.55$,

$P=0.012$). However, this relationship was not significant when either year was analysed separately.

Fifty-seven of 81 marked pairs separated naturally during the course of the study. In 40 of these 57 pairs, separation was the result of male disappearance (19 cases), female disappearance (12 cases), or the disappearance of both individuals (9 cases). We use the term disappearance to indicate that an animal was never again seen, despite intensive searches that included grid and off-grid areas. Males and females disappeared from territories at equal rates (binomial test: NS). In the remaining 17 pairs, separation was the result of the male abandoning the female and moving to a new (not previously occupied) territory (16 cases) or the female abandoning the male and moving to a new territory (1 case). Males moved to new territories significantly more often than females (binomial test: $P=0.002$).

Remating

There were no between-site differences in the time required to remate, so we pooled data across sites. We removed one individual from each of 32 pairs, resulting in 15 single females and 17 single males. Ten of 15 single females remained on their original coral heads and were joined by new males ($\bar{X} \pm \text{SD}=8 \pm 5$ days to remate). Two single females remained on their original coral heads but did not form pairs, two females moved and joined previously solitary males, and one female disappeared. Among the 17 single males, 11 remained on the original coral heads and were joined by new females ($\bar{X} \pm \text{SD}=7 \pm 7$ days to remate), three males remained on their original coral heads but did not form pairs, one male moved and joined a solitary female, and two males disappeared. All experimentally widowed fish had an average \pm SD of 3 ± 2 neighbours (range 0–6) within a 6-m radius of their territory before the experiment.

Responses of Pair Members to Staged Territorial Intrusions

The behaviour of male and female residents towards conspecific intruders depended strongly on the sex and size of the intruder. Residents bit intruders of the same sex at higher rates than intruders of the opposite sex (Fig. 2a). Bite rates of resident males and females differed significantly between intruder types (Friedman's ANOVA: male residents: $\chi^2_3=13.6$, $P=0.004$; female residents: $\chi^2_3=15.3$, $P=0.002$). Residents also performed threat displays towards intruders of the same sex more often than intruders of the opposite sex (Fig. 2b). As with bite rates, threat-display rates of resident males and females differed significantly, depending on the type of intruder (male residents: $\chi^2_3=11.9$, $P=0.008$; female residents: $\chi^2_3=17.7$, $P=0.001$). Chase rates by residents (Fig. 2c) also differed significantly, depending on the sex and size of the intruder (male residents: $\chi^2_3=14.9$, $P=0.002$; female residents: $\chi^2_3=23.5$, $P<0.001$). Chases often followed threat displays, while threat displays often preceded biting, indicating that these behaviours were not independent.

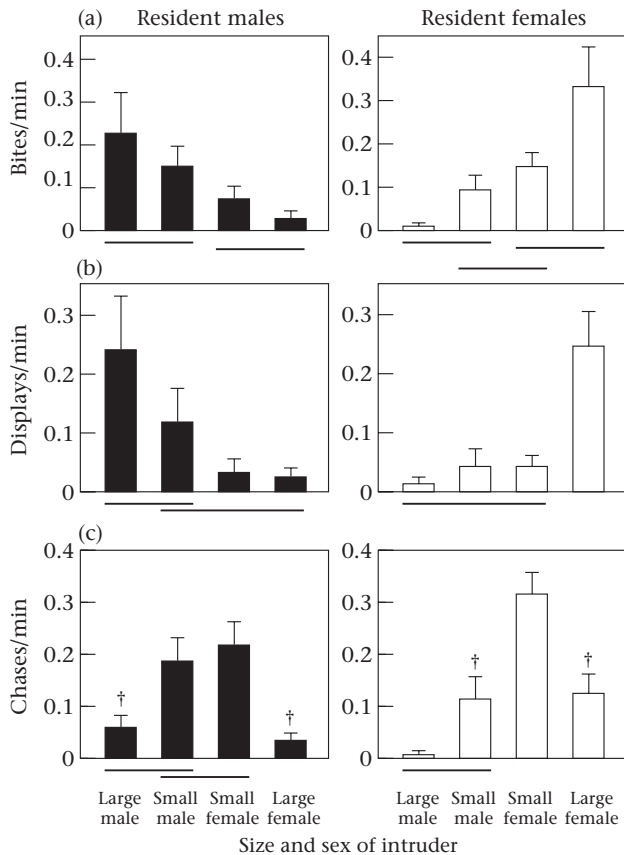


Figure 2. Behavioural responses of 12 paired male and female *G. evelynae* to four types of conspecific intruders experimentally introduced to the pair's territory. Horizontal lines below the abscissa and daggers above bars denote nonsignificant ($\alpha=0.05$) differences between adjacent and nonadjacent treatments, respectively, in a posteriori multiple comparisons (Tukey-type test). (a) Bite/min ($\bar{X} \pm SE$) of resident males and females. (b) Threat display/min ($\bar{X} \pm SE$) of resident males and females. (c) Chase/min ($\bar{X} \pm SE$) of resident males and females.

In general, large intruders of the same sex were most vigorously attacked. Large intruders of the opposite sex were usually approached, inspected and ignored. Small intruders were generally chased and expelled by both residents, regardless of the intruder's sex.

Responses of Unpaired Females to Staged Territorial Intrusions

Unpaired females responded to large male and female intruders in a pattern similar to that of paired females: female intruders were attacked more often than male intruders (Fig. 3). Bite rates differed significantly between male and female intruders (Wilcoxon two-sample test: $W=21$, $N=6$, $P<0.05$). However, the small sample size was insufficient to detect any potential sex-specific differences in either threat display rate or chase rate (threat display rate: $W=19$, $N=6$, NS; chase rate: $W=10$, $N=6$, NS).

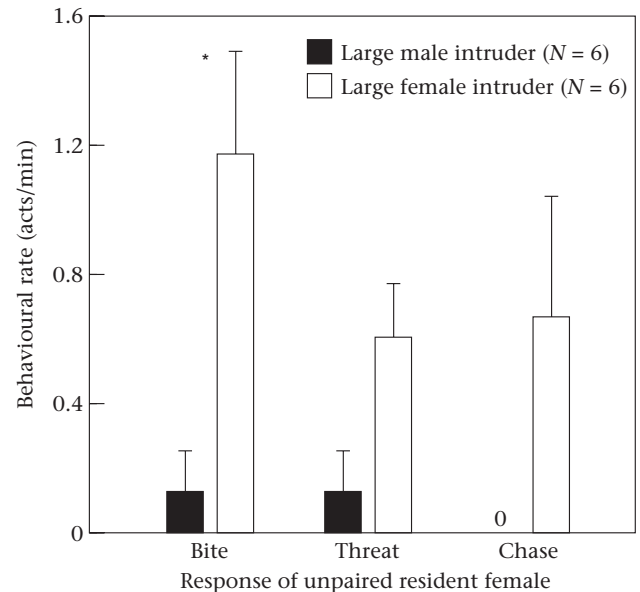


Figure 3. Behavioural responses ($\bar{X} \pm SE$) of six unpaired female *G. evelynae* to large male and female conspecific intruders experimentally introduced to the female's territory. *Denotes a significant difference ($P<0.05$) in the resident female's response to intruder types.

Distribution of Paired and Unpaired Gobies in Relation to Suitable Habitat

Using the Clark & Evans method of nearest-neighbour analysis, we determined the index of aggregation (R) associated with the distribution of coral heads in each survey grid. Note that R can vary from a minimum of zero (perfectly clumped) to a maximum of 2.15 (perfectly uniform); an R of 1.0 indicates a perfectly random pattern. Spatial distribution differs significantly from random if $|z| > 1.96$ (Krebs 1989). Randomly distributed habitat is by itself unlikely to promote monogamy. The distribution of inhabitable coral heads (Fig. 4) was random within the survey grid at both sites (Saba Cay: $R=0.99$, $z=-0.49$, $N=595$, NS; Flat Cay: $R=1.05$, $z=1.84$, $N=487$, NS). The distribution of female-occupied coral heads (Fig. 4) was random within the survey grid at Saba Cay in both years (1991: $R=1.12$, $z=0.63$, $N=11$, NS; 1992: $R=1.31$, $z=1.89$, $N=14$, NS). The distribution of female-occupied coral heads at Flat Cay was random in 1991 ($R=1.23$, $z=1.77$, $N=19$, NS), but marginally uniform in 1992 when the survey grid was enlarged ($R=1.23$, $z=2.05$, $N=25$, $P<0.05$).

Male nest crevices were typically located in patches of dead coral less than 1 m from the male's normal resting spot ($\bar{X} \pm SD=0.38 \pm 0.37$ m, $N=24$). When territorial aggression was observed, resident males did not pursue intruders farther than 0.5–1.0 m from their resting spot. These observations suggest that male territories had a maximum radius of approximately 1 m. Assuming male territories are roughly circular and centred on the male's resting spot, there were no male territories that included more than one female-occupied coral head (Fig. 4a, b).

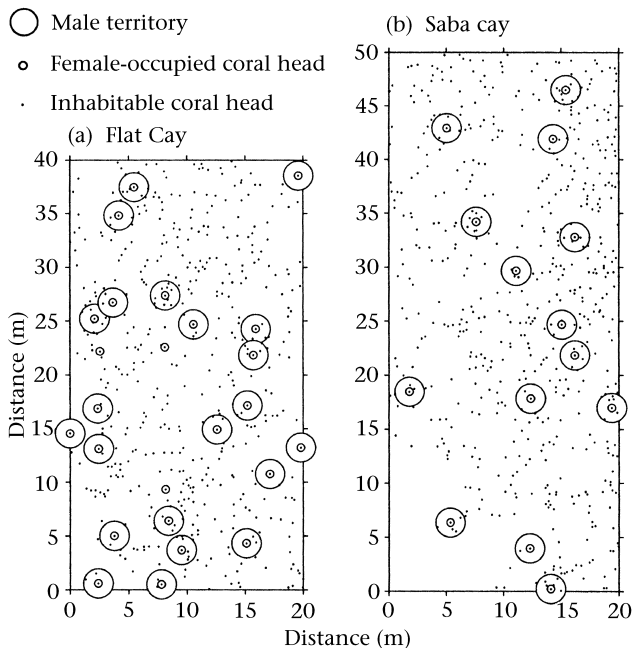


Figure 4. (a) Scaled map of all uninhabitable coral heads, and all coral heads occupied by *G. evelynae* within the survey grid at (a) Flat Cay and (b) Saba Cay, St Thomas, September 1992. At Flat Cay, 22 of 25 occupied coral heads contained one male–female pair; the remaining three each had a single female. The distribution of uninhabitable coral heads was random and the distribution of females was marginally uniform (see text). At Saba Cay, each occupied coral head contained one male–female pair. The distribution of uninhabitable coral heads and the distribution of females was also random (see text). At both sites male territories contained only one female-occupied coral head and from zero to seven unoccupied but uninhabitable coral heads. Male territories seldom overlapped.

DISCUSSION

Field observations revealed that *G. evelynae* is monogamous. Both male and female are site tenacious on the same coral head, and both contribute to territorial defence. Specifically, males exclude rival male intruders, and females guard against female intruders. Within a pair's territory, the male alone locates and defends a suitable crevice or other shelter where eggs are laid. Nest site defence is facilitated because territories are small, discrete sites that occur discontinuously within a broader area containing coral reef or rubble. In addition to territorial defence, a male and female typically maintain a prolonged relationship, which persists during multiple spawning cycles.

Given the tenacious and prolonged mutual defence of territory, we suggest that *G. evelynae* pairs may be genetically as well as socially monogamous. This inference is supported in part by observations that egg deposition and immediate fertilization occurs within a crevice that is well defended when eggs are present, presumably precluding any opportunity for rival males to fertilize the ova. We also note that both the female and the male are extremely site tenacious, and that temporary movements (even

between nearby territories) have never been observed. Furthermore, most adults on the study sites were paired, with relatively few territories occupied by a single adult. Nevertheless, unrelated offspring have been found in nests of apparently monogamous pairs for a variety of vertebrates (Gowaty 1997; Stamps 1997). Thus, DNA tests of egg clutches, putative parents and nearby adults are required to resolve the issue of genetic monogamy in *G. evelynae*.

In any case, these gobies are clearly serially monogamous. Pair abandonment occurred as some individuals sought new mates, or a new territory where a different mate would subsequently be acquired. The duration of the spawning season for these gobies is lengthy, relative to a single spawning cycle of 6–8 days. Thus, it is not surprising that paired individuals sometimes found new partners. The critical finding, however, is that adults are monogamous, being much more likely to form a prolonged relationship in a pair (averaging 4–6 spawning cycles), rather than defend a territory alone.

Abundant 'Unused' Habitat

In goby species for which monogamy has been documented, the availability of suitable territories does not appear to be a limiting factor (Reavis 1997a, b; Reavis & Barlow 1998). The significance of an excess abundance of potential nesting sites is that every male in a population presumably can occupy and defend a nesting territory. This conclusion is supported by the lack of any observations of roving unmated males. Furthermore, because females require access to a nesting site and male parental care, when males are highly dispersed females are essentially constrained to associate with a single male. Although gobies are not well known phylogenetically (Nelson 1994), monogamy has apparently evolved multiple times (Thresher 1984). In each monogamous species that has been studied, territory availability was not a limiting factor. For *G. evelynae* in particular, we have documented a substantial amount of 'unused' habitat within our study sites.

If unused habitat is abundant, what limits the adult population size of these gobies? If settling goby larvae have strong preferences for particular types of coral heads, and these coral heads are not abundant, habitat selection could limit population size (Wilson & Osenberg 2002). What about interactions such as competition and predation? We are unaware of any evidence that cleaner shrimp and cleaner gobies compete, and we can rule out competition with cleaner wrasses, as these specialized fish do not occur in the Caribbean. In addition, juvenile or subadult gobies were not seen on the study sites, and thus did not directly influence the adults. Reavis (1997a, b) suggested that predation on adults may have limited populations of the monogamous Indo-Pacific goby, *Valencienna strigata*. Presumably as a consequence of predator-limited population size, each male *V. strigata* had sole access to a suitable breeding territory. It is possible that *G. evelynae* is vulnerable to predation by invertebrate predators such as stomatopods (see

Hixon 1991): 49 of 162 individuals in the 81 pairs we monitored for pair stability disappeared, presumably due to predation. We cannot say whether predation by invertebrates is sufficient to reduce adult populations to the level where excess suitable habitat is available. In any case, predation by other fish is unlikely to account for the limited population size of *G. evelynae* in our study area. Unlike *V. strigata*, *G. evelynae* are obligate cleaner fish that remove parasites from host fish attracted to the cleaning station, which is within the territory of a given pair. As with other cleaner species, *G. evelynae* most likely receive a measure of immunity from predation in return for the service of removing parasites from hosts, many of which are predators (Darcy et al. 1974; Carr & Hixon 1995).

What factors, in addition to predation on adults, might account for the 'unused' habitat within our study sites? As *G. evelynae* rely on visits from parasitized (host) fish for most of their food, host fish behaviour may influence the number of cleaning stations that can be supported within a given area. Interactions between cleaner fish and their hosts are not well understood (Arnal et al. 1999), however, and we know of no documented cases in which host availability or behaviour limits the density of goby cleaners. This hypothesis could be tested, however, by introducing *G. evelynae* pairs from other populations to vacant territories on our study sites. If hosts were limited, introduced pairs should attract relatively few hosts, and host visits at nearby established stations might decline. This scenario is not complicated by the presence of juvenile or subadult gobies, which are not seen at adult cleaning stations (personal observation).

Alternatively, the abundance of adult *G. evelynae* may be limited indirectly by the aggression that highly territorial damselfish (*Stegastes* spp.) direct towards host fish. These damselfish were abundant in our study grids. Moreover, Arnal & Cote (1998) found that, when damselfish and cleaner goby territories overlapped, gobies were visited by significantly fewer host species and by fewer individual hosts than were gobies outside damselfish territories. Also, gobies within damselfish territories spent less time attending clients and experienced decreased feeding rates. These effects appear to be due to the repeated aggression that territorial damselfish show towards fish intruding on their territories to visit cleaning stations (Arnal & Cote 1998). Thus, if cleaning stations within a damselfish territory are relatively unprofitable, damselfish abundance may limit the number of profitable cleaning stations on a reef, and thus limit *G. evelynae* abundance. This hypothesis could be tested by: (1) quantifying the effects of damselfish territorial defence in relation to host visits to goby territories, and (2) conducting a territorial damselfish removal experiment to document changes in host visits to cleaning sites formerly within damselfish territories.

Perhaps the most plausible explanation for the abundance of unoccupied habitat is a low abundance of goby larvae (i.e. 'recruitment limitation', sensu Doherty 1981, 1983). In this situation, larval numbers are insufficient to saturate the habitat due to low regional fecundity and/or high mortality of developing larvae in the plankton. Recruitment limitation alone could account for habitats

that are undersaturated with gobies. This hypothesis could be tested by collecting and transplanting newly settled *G. evelynae* to suitable coral heads within our study grids and following their fate. If transplanted individuals survive and reproduce in the study grids, then host fish behaviour or damselfish aggression are unlikely to account for the abundance of suitable, unoccupied habitat. Instead, the natural supply of *G. evelynae* larvae to these sites is probably insufficient to saturate available habitat.

Whatever the cause(s) of abundant unused habitat, one of the main behavioural factors promoting the monogamous bond in *G. evelynae* was strong, sex-specific aggression. Resident males and females directed most aggression towards conspecific intruders of the same sex and tended to ignore those of the opposite sex. Same-sex intruders were attacked even when the intruder was larger than the resident, and when only a single female was defending a territory. This pattern of aggression is consistent with defence of resources, which could include a food-rich territory and sole access to a mate. Similar patterns of sex-specific aggression have been observed in some monogamous species of butterflyfish (Chaetodontidae) and angelfish (Pomacanthidae), and appear to promote and maintain long-term pair bonds in these species (Hourigan 1989; Hourigan et al. 1989). Most other studies of pairing in coral reef fish have not examined aggression by territorial fish in sufficient detail to test for sex-specific aggression, either because the sex of the interactors was unknown (e.g. Lassig 1976; Driscoll & Driscoll 1988) or the response of territory residents to conspecific intruders was not measured (e.g. Tricas 1989). However, in one of the most thorough studies to date, Fricke (1986) induced intrasexual aggression in a monogamous butterflyfish using caged intruders of known sex. Based on these trials, and on observations of territorial disputes between tagged individuals, Fricke concluded that sex-specific aggression results in mutual mate-monopolization. However, Fricke concluded that (1) aggression was independent of reproduction because pairs comprised of two juveniles or two same-sex adults were occasionally observed, and (2) pairing occurred because two individuals were necessary for territory defence. This situation does not appear to be the case for *G. evelynae* as juveniles did not form pairs and single adults that lost their partners successfully defended their territories. Whether or not mates are transitorily guarded in *G. evelynae*, strong sex-specific aggression results in mate monopolization and promotes long-term pairing.

It is tempting to view joint male-female territorial defence as a cooperative division of labour beneficial to both partners. However, a partnership of this kind is not necessarily the best possible reproductive strategy for both sexes. Certain patterns of aggression, such as those we observed in *G. evelynae*, may generate a conflict of interest between the sexes. Male *G. evelynae* cannot move freely among females while guarding a nest and cannot form a harem at the nest site because of the aggressive behaviour of the resident female. As a result, the male is likely to be the reproductively manipulated sex, if such a conflict exists in this species.

Territorial Aggression

Sex-specific territorial aggression also was expressed in a context separate from mate guarding by single females that defended individual territory. When we introduced males or females to the territory of a single female, the territorial female was most aggressive towards intruding females and tended to ignore male intruders. Aggressive behaviour from a single individual is inconsistent with simple mate monopolization. This pattern suggests an active defence of other resources associated with the territory, including food, shelter and crevices used for oviposition. We never observed females defending crevices, however, and males defended these oviposition sites only when an egg mass was present. Other observations suggest that food availability is a likely resource that is actively defended. This food resource cannot be monopolized or guarded because host fish move freely among the territories of several gobies (personal observation). However, some goby territories appear to be of better quality than others: gobies in some territories spend more time cleaning and clean more large fish than those in other territories (Harding 1993). This variation is apparently due to an uneven distribution of host fish. For example, during 20 h of monitoring gobies in territories adjacent to large, semipermanent aggregations of *Chromis multilineata* and *C. cyanea* damselfish, over 80% of all goby cleaning acts involved these two damselfish species as hosts (Harding 1993). Therefore, we hypothesize that food-rich cleaning stations may be defended by the resident female, whether that female is single or paired.

Interterritorial Movement, Remating and Size-assortative Mating

Tenacious site defence does not prevent movement between territories and remating by abandoned mates. When we experimentally separated pairs of gobies, single fish were often able to remate within a few days. This rapid remating could suggest that there was a mobile pool of adults that filled vacancies created by pair separation. However, observations of single females that obtained new mates often revealed that a female's new mate had previously been paired with a different female. Thus, remating can occur at the expense of another pair's separation. When pairs separated due to the abandonment of one partner by the other, males deserted females far more often than females deserted males. However, we do not know how often males deserted females to mate with another female. In addition, in the mate removal experiment we could not determine whether migrants had been paired or solitary prior to immigration. Even if desertion uncommonly leads to remating, this behaviour still represents a potentially significant method by which a male could achieve higher reproductive success if the shift enabled him to mate with a larger female or move to a more resource-rich territory.

When remating occurred, size-assortative mating was still present between females and second males, but not between males and second females (Harding 1993). This pattern suggests that females actively choose a second

mate on the basis of size (cf. McKaye 1986), whereas males do not. The advantage of mating with a large female is that larger females typically produce more ova (Potts & Wootton 1984). Another consideration, however, is the expeditious production of a new clutch. Males may be willing to accept a smaller female as a mate when a larger female is unavailable. This tactic may be part of a larger strategy in which there is the possibility of deserting the small female in favour of pairing with a larger female on a new territory. Thus, the combination of highly aggressive territorial defence with the possibility of up-grading partners and territories presents a surprisingly complex picture of the reproductive interactions among these gobies.

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Erratum

Harding, J. A., Almany, G. R., Houck, L. D. & Hixon, M. A. 2003. Experimental analysis of monogamy in the Caribbean cleaner goby, *Gobiosoma evelynae*. *Animal Behaviour*, **65**, 865–874.

It is regretted that the island where the study took place was misidentified in the abstract. A corrected abstract is given below.

Abstract

To investigate factors promoting monogamy, we studied the reproductive behaviour and ecology of the monogamous Caribbean cleaner goby at two sites near St Thomas, U.S. Virgin Islands. We assessed mate fidelity and the amount of time required to acquire a new mate in experimentally widowed males and females. We also measured behavioural responses of pair members and

single females to experimentally introduced conspecific intruders of both sexes. Finally, we evaluated the distribution of suitable habitat relative to existing goby territories. We found that pairs often separated due to males abandoning females and moving to a new territory, that most widowed fish soon acquired a new mate, and that there was strong aggression towards large same-sex intruders. We also found an abundance of suitable, unoccupied habitat. We conclude that pairs are maintained by intrasexual aggression that is related to both mate availability and other resources, such as high-quality, food-rich cleaning stations. Combined with observations of frequent pair separation, interterritory male movement, and relatively rapid remating by both sexes, these results suggest a complex mating system that is best classified as serial monogamy.