

# Selection of diurnal refuges by the nocturnal squirrelfish, *Holocentrus rufus*

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**Abstract** We examined the diurnal refuges occupied by the nocturnal squirrelfish, *Holocentrus rufus*, to describe refuges and the behavior associated with their use and to determine which, if any, refuge characteristics were selected. We tagged 21 *H. rufus* on two sites on a fringing reef in Barbados, West Indies, identified the refuges they used ( $n = 57$ ), measured ten characteristics of each refuge and the surrounding microhabitat, and monitored their refuge use for 4 weeks. To evaluate refuge selection, we measured the same characteristics on a comparable number of unused potential refuges ( $n = 67$ ) on the same reefs and used classification tree models to determine which characteristics separated used from unused refuges. Each fish used 1–9 refuges, which did not overlap among individuals and were defended against intrusion by conspecifics and some heterospecifics. Fish with more than one refuge frequently moved among them. There was strong site fidelity with no immigration of untagged fish or emigration of tagged fish on either reef during the study period and no additional refuges being occupied over the 4-week period. Refuges were primarily holes, open at one or two ends, which varied in size, distance from the reef edge, entrance orientation, and vertical relief at the entrance. Holes used as refuges differed significantly

from unused holes mainly in characteristics related to the vertical position of their entrance, but the classification tree models differed for the two sites. This study provides the first detailed information on characteristics of daytime refuges used by a nocturnally active reef fish and the first evidence of selectivity of refuges. It suggests that the abundance and characteristics of holes on reefs could influence the density of *H. rufus* on natural reefs.

**Keywords** Caribbean · Coral reef fish · Habitat selection · Holocentridae · Microhabitat · Shelter

## Introduction

Many fish species spend all or much of their time closely associated with physical structure, for example within clusters of algae, plants or coral, under rocks or in shells, caves, holes or the interstitial spaces of rubble. These structural microhabitats, often referred to as “refuges” (Krause et al. 2002) or “shelters” (Steele 1999), may reduce predation risk as well as providing sites for feeding and reproduction and offering protection from strong currents (Krause et al. 2002). Among coral reef fishes, refuge use is very common, often changing over the diel cycle. Species from several families that forage at night in the water column or on open substrate retreat to refuges on the reef during the day (e.g., Apogonidae, Holocentridae, Mullidae and

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Pempheridae: Wyatt 1983; Marnane 2000; Meyer et al. 2000; Annese and Kingsford 2005). Similarly, species from many day-active families use refuges at night (e.g., Acanthuridae, Chaetodontidae, Labridae, Pomacentridae and Scaridae: Collette and Talbot 1972). Other species spend most of their time in refuges, both day and night (e.g., many Chaenopsidae and Muraenidae: Smith and Tyler 1972; Clarke and Tyler 2003; Young and Winn 2003; Gilbert et al. 2005).

Holes, crevices, and caves are an important component of habitat quality on coral reefs. The abundance of many species is related to availability of holes on artificial reefs made of synthetic materials (Hixon and Beets 1989, 1993; Buchheim and Hixon 1992; Caley and St-John 1996; Lindberg et al. 2006) or corals (Holbrook and Schmitt 2002; Almany 2004) as well as natural reefs (Roberts and Ormond 1987; Friedlander and Parrish 1998). The presence of holes may explain some of the widespread relationship between the structural complexity of natural reefs and fish abundance (Luckhurst and Luckhurst 1978a; Roberts and Ormond 1987; McCormick 1994; Connell and Kingsford 1998). However, holes are not always a limiting resource (Robertson and Sheldon 1979; Robertson et al. 1981). The importance of holes as a resource is likely to depend on the type of refuge used, the degree of selectivity in relation to availability, and the extent to which refuges are shared or defended. Previous studies have demonstrated competition for refuge holes among coral reef fish (Shulman 1985; Buchheim and Hixon 1992), but there is surprisingly little information about the characteristics of holes on coral reefs or how they are used by fishes (but see Clarke 1994; Clarke and Tyler 2003). This may be due, in part, to the diversity of hole types and the challenge of quantifying their characteristics. Most studies of refuge hole characteristics have focused on size (e.g., height, width, length, diameter, and volume: Buchheim and Hixon 1992; Clarke and Tyler 2003; Young and Winn 2003; Almany 2004; Forrester and Steele 2004), although height of refuge above reef substrate (Clarke and Tyler 2003), various qualitative characteristics (Robertson and Sheldon 1979; Clarke and Tyler 2003) and substrate around the refuge (Clarke and Tyler 2003) have also been considered. Most previous studies of refuge hole characteristics in coral reef fishes have examined a limited set of characteristics, and none

has considered the relative importance of multiple characteristics in a multivariate analysis.

We studied the nocturnally active squirrelfish, *Holocentrus rufus* (Walbaum), which uses diurnal refuges in the form of holes and caves (Winn et al. 1964; Collette and Talbot 1972). Our primary goal was to describe quantitatively the characteristics of holes used as refuges and to determine whether they were significantly different from unused refuge holes. In addition, we examined refuge use behavior, including the number of refuges used, whether individuals consistently used the same refuge(s), whether refuge size or number was related to fish size, and whether there was evidence for territorial defense of refuges through exclusive use and aggressive responses to intruders.

## Materials and methods

### Study site and species

The study was conducted at two sites (S1, S2) in the spur and groove zone of Heron Bay Reef, a fringing reef on the west coast of Barbados, West Indies (13°11'48"N, 59°38'37"W). Heron Bay Reef extends approximately 125 m offshore to a maximum water depth of 6.2 m and has a width of 300 m along the shoreline. S1 was an isolated patch reef (area: 92 m<sup>2</sup>, mean depth 4.9 m) and S2 was a tip of a spur (area: 136 m<sup>2</sup>, mean depth 4.7 m). The mean height of S1 above the surrounding sand (mean  $\pm$  SD = 50.9  $\pm$  45.4 cm, max = 150 cm) was less than that of S2 (66.7  $\pm$  39.1 cm, max = 190 cm). Both sites were similar in substratum. Main substratum types on S1 were rock covered with turf algae (70%), eroded dead coral (16%), and live coral [9%, predominantly massive starlet coral, *Siderastrea siderea* (Ellis and Solander), and finger coral, *Porites porites* (Pallas)]. On S2, main substratum types were rock with turf algae (67%), eroded dead coral (22%), and live coral [12%, mostly *P. porites* and mustard hill coral, *Porites astreoides* (Lamarck)]. Both sites were used concurrently for a project on density-dependent movement of longfin damselfish, *Stegastes diencaeus* (Jordan and Rutter) (K. Turgeon and D. Kramer, unpublished observations). The damselfish project involved considerable activity (three divers, about 4 h per day) during the entire study period and

damsel fish removal during part of it. We did not collect squirrelfish observations during or for at least 10 min after habitat measurements or damselfish removal.

Like other members of the family Holocentridae, *H. rufus* is a reddish nocturnal fish that spends the day in or near crevices and caves or under overhangs (Böhlke and Chaplin 1993). This species forages at night on benthic crustaceans and is found at depths to 100 m (Randall 1967; Gladfelter and Johnson 1983; Wyatt 1983). Known predators include the trumpetfish, *Aulostomus maculatus* (Valenciennes), some Serranidae [*Epinephelus striatus* (Bloch), *Mycteroperca venenosa* (Linnaeus), and *Cephalopholis cruentata* (Lacépède)] and Lutjanidae [*Lutjanus jocu* (Bloch and Schneider) and *L. mahogony* (Cuvier)] (Randall 1967), as well as the spotted moray, *Gymnothorax moringa* (Cuvier) (Young and Winn 2003). Site fidelity to reefs has been reported for this species (Luckhurst and Luckhurst 1978b; Chapman and Kramer 2000), but fidelity to specific refuges has not been examined previously.

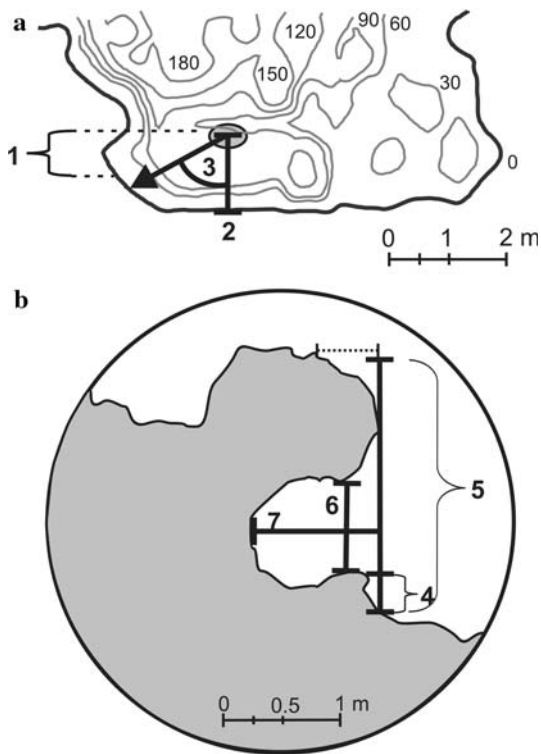
### Study design

We collected data using SCUBA from 14 June to 22 August 2005 between 10:00 and 17:00 h. We captured all *H. rufus* on S1 ( $n = 16$ , from 14 to 17 June) and S2 ( $n = 5$ , on 24 July) using baited hook and line or modified cast net, brought them to the surface, and measured them to the nearest mm [total length (TL)]. We tagged each fish with a numbered T-bar anchor tag (Floy tag) inserted into the muscle at the base of the dorsal fin. After tagging, we immediately released each fish in the refuge where it had been captured. From 19 to 23 June, 2 days after tagging on S1, and from 25 to 26 July, 2 days after tagging on S2, we observed each tagged individual for a 10-min focal observation to determine the position of all the refuges it used. Preliminary observations on both sites suggested that a 10-min period was sufficient to detect all diurnal refuges, and this was confirmed during eight 18- to 40-min observations of *H. rufus* on the same reef in January 2006 (D. L. Kramer, unpublished data). During the 10-min focal observation, we also recorded which species attacked and were attacked by the focal *H. rufus* and any others fishes present in the refuges used by the focal individual. After we completed 10-min

focal observations for all fish, we checked each fish on 22 of 28 days on S1 (23 June to 20 July) and 21 of 28 days on S2 (26 July to 22 August), recording whether the tagged fish were present and whether they were using the refuges previously identified during the focal observation.

### Refuge characteristics

After each 10-min focal observation, we recorded nine quantitative and three qualitative characteristics of each refuge used by the focal individual. We chose characteristics related to shape, dimensions and number of entrances of the refuge because these might affect its value in avoiding a pursuing predator as well as exposure to light. We chose characteristics related to the vertical relief around the entrance because these might affect ability of an occupant to detect a predator or to be detected by one. We chose characteristics related to height above the substrate, direction faced by the entrance, and distance from the edge of the reef because these might affect exposure to transient predators, access to nocturnal feeding sites on the reef, and exposure to onshore waves. The quantitative characteristics were the height of the center of the refuge entrance above the sand substrate adjacent to the reef, the shortest distance from the refuge entrance to the reef edge, the orientation of the refuge entrance in the horizontal plane in relation to the shortest distance to the reef edge, the height of the bottom of the refuge entrance above the reef substratum, the vertical relief of the reef at the refuge entrance, the width, height, and area of the refuge entrance, and the length of refuge (Fig. 1). For refuges with more than one entrance, we only measured the largest one and used that entrance for the measurement related to refuge positioning. We recorded most of these characteristics underwater, but we determined reef height above sand at the refuge entrance and the shortest distance from refuge to reef edge using georeferenced maps of the sites that we created (see below). We measured the orientation of each refuge (direction faced by the middle of refuge entrance) underwater, then calculated the difference (in degrees) from the line representing the shortest distance to the reef edge on the map. For measures of the vertical positioning of the entrance, we held a graduated (cm) PVC pipe vertically against the entrance. We then measured the distance from the

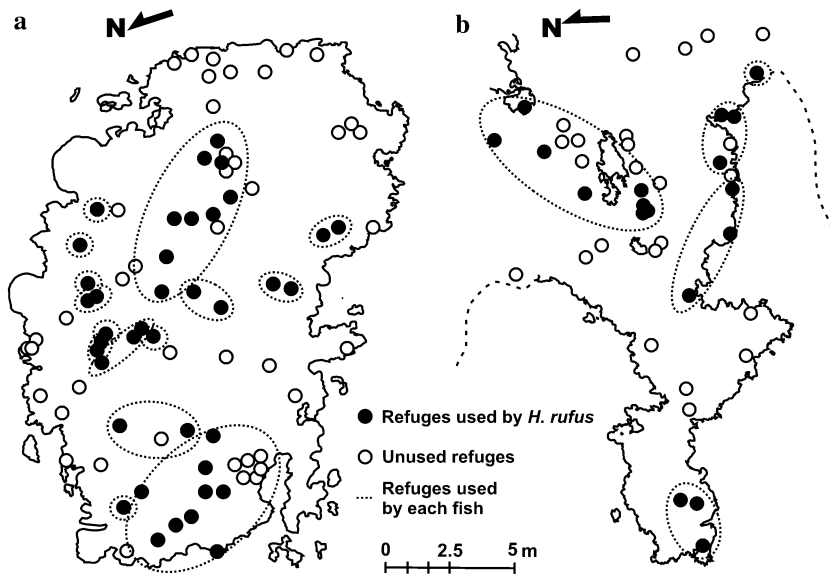


**Fig. 1** Schematic diagram of seven quantitative habitat measurements used to characterize the refuges occupied by *H. rufus* and unused potential refuges. **(a)** Overhead view of a refuge entrance (gray oval) and surrounding area plotted on a map of part of S1: (1) height of refuge above sand (contour lines, cm), (2) shortest distance from refuge to reef edge (cm), and (3) orientation of refuge entrance relative to nearest reef edge (degrees). **(b)** Close-up lateral view of the same refuge: (4) height of refuge above reef substrate (cm), (5) vertical relief of reef at the refuge [within 50 cm (dotted line) of the measuring pipe held vertically, cm], (6) refuge height, and (7) refuge length (cm). The refuge width and the calculated entrance area are not shown

lower edge of the entrance down to the point where the lower end of the pipe contacted the reef (height of entrance above reef substratum, cm). We also measured the distance from the bottom of the pipe up to the highest point on the reef within 50 cm of the vertical pipe (vertical relief of the reef at the refuge entrance, cm). We calculated the refuge entrance area by taking the entrance width and height and applying the formula for the area of an ellipse ( $0.7854 \text{ height} \times \text{width}$ ) (Aitken and Martin 2004). We measured the refuge length (cm) from the entrance to the back wall for refuges with a closed end and from one entrance to the other for refuges with an open end. The qualitative characteristics were the refuge structure

type (overhang, hole, space under platform), the closure of the refuge (open versus closed ends) and the main substratum type (fire coral, dead coral, live coral, bare rock or sponge) in a 15 cm-band around the refuge entrance. Overhangs consisted of spaces under coral heads open at the front and sides but closed at the back, holes were enclosed spaces with only one or two openings, and spaces under platforms had multiple openings on the sides. After we had identified and characterized all the refuges used by *H. rufus* on each site, we selected and characterized, using the same procedures, a similar number of potential refuges that were not used by *H. rufus*. We chose these refuges by zigzagging back and forth over the sites and selecting any potential overhang, hole or space under a platform that measured at least  $4 \times 4 \times 11$  cm in any dimension (Fig. 2). We chose this criterion to include all holes large enough to permit entry of at least some *H. rufus*. Although we did not measure all holes and overhangs, we estimated that the used refuges and unused potential refuges together represented at least 75% of the available refuges of appropriate size on each study site.

Georeferenced maps built for the *S. diencaeus* project (K. Turgeon and D. L. Kramer, unpublished data) were used to plot refuge position and estimate several characteristics (see below). We created a  $1 \times 1$  m grid on S1 and S2 using a 15-m nylon rope graduated every meter, attached to two 3-m PVC pipes that we held vertical. We placed a measuring tape along the long axis of the site and determined the orientation using a compass to facilitate the geographical orientation of the map. We gradually moved the pipes every meter along the measuring tape, perpendicularly to the shore to cover the entire surface of each site. We positioned the graduated rope at a fixed distance above the sand bottom and we took five measurements of distance from the rope down to the reef. In addition, we took a digital photograph of the substratum in each  $1 \times 1$  m cell. We calculated reef height above sand by subtracting the reef-to-rope values from the height of the rope above the sand. We assembled the digital pictures in a graphic software (Corel Photopaint 10) and georeferenced them using GIS software (MapInfo Professional 6.5). We plotted every used and unused refuge on the georeferenced maps using reference points such as uniquely shaped reef structures and coral



**Fig. 2** Map of the two study sites on Heron Bay reef, Barbados. (a) S1 was a patch reef with a surface area of 92 m<sup>2</sup>. A total of 39 refuges occupied (filled circles) by 16 *H. rufus* and 43 potential but unoccupied refuges (open circles) were characterized. (b) S2 was part of a spur tip, contiguous with the

main fringing reef with a sampled surface area of 136 m<sup>2</sup>. A total of 18 refuges occupied (filled circles) by five *H. rufus* and 24 potential but unoccupied refuges (open circles) were characterized. The dotted ellipses enclose the refuges used by each individual

colonies. Then, for each refuge, we determined the values for the reef height above sand, the shortest distance from refuge to reef edge, the orientation relative to that shortest distance, and the maximum distance between refuges used by each fish using the GIS software.

Statistical analyses

We had to transform some variables (shortest distance to reef edge and orientation relative to reef edge with square root; vertical relief, width, height, entrance area, and length of refuge, fish length, number of refuges, and the maximum distance between refuges with log<sub>10</sub> in order to achieve a normal distribution. Only height above reef substrate could not be normalized. We used univariate *t*-tests with sequential Bonferroni correction (Holm 1979) to evaluate whether the quantitative refuge characteristics differed between and within sites. To evaluate whether the qualitative characteristics differed between and within sites, we used chi-square tests. We used linear regressions on the transformed data to determine whether the size and number of refuges were related to body size. For these regressions, we

combined sites because there was little variation in body size within sites. Variability of descriptive statistics is indicated by the SD, unless otherwise indicated.

Refuge selection models were built using STATISTICA 6.0 and SYSTAT 11.0 for Windows. To determine whether there was selection for specific refuge characteristics (used versus unused refuges), we used classification trees (CT; Breiman et al. 1984) to build models based on the 10 refuge characteristics. CT models offer a flexible and simple alternative for modeling complex ecological relationships (De’ath and Fabricius 2000), producing graphical trees that are easy to visualize and interpret. CT models are also able to capture complex interactions between explanatory variables, can handle missing values, can model a mix of continuous and categorical variables (Breiman et al. 1984; De’ath and Fabricius 2000), and are more parsimonious than other classification methods for equivalent performance (Turgeon and Rodríguez 2005).

We built separate CT models for each site and applied them on their own data set (calibration on S1 and S2) so that we could later evaluate model transferability. Prior to building the classification

trees, we used the tolerance measured from a GLM test (one minus the squared multiple correlation between one characteristics and the remaining ones) to detect the multicollinearity between the refuge characteristics (Afifi et al. 2004).

Height of refuge above sand and shortest distance to reef edge showed multicollinearity as indicated by a tolerance of 0.3. We excluded distance from reef edge from the models because it explained less model variation than height of refuge above sand (Legendre and Legendre 1998). Beginning with the entire data set (called the root node), the algorithm (C&RT; Breiman et al. 1984) examined all possible splits of the response variable (used and unused refuges) for each possible value of the refuge characteristics, and selected the candidate split (the “splitting value”) that maximized the homogeneity within the two resulting subgroups, using the Gini index as a measure of goodness of fit. CTs were pruned using the cost-complexity parameter (CP) in STATISTICA 6.0 to eliminate splits that did not improve the fit by at least the value of CP, fixed at 0.01 in this study (Atkinson and Therneau 2000). Tenfold cross-validation was used to select optimal trees (Atkinson and Therneau 2000; De’ath and Fabricius 2000). Fifty sets of tenfold cross-validation were run for each site, and the most frequently occurring tree size was chosen (De’ath and Fabricius 2000). In CT models, the contribution of refuge characteristics was evaluated with the proportional reduction in error (PRE, a measure of the variability accounted for by the splits associated with each microhabitat variable similar to  $R^2$ ). Then, we validated the models for their transferability by using crossover field tests, which involve applying the model calibrated on S1 to the data of S2 and vice-versa (validation on S1 and S2).

To assess accuracy of both calibration and validation models, we obtained from confusion matrices the correct classification rate (CCR; percentage of all cases correctly predicted), the sensitivity (percentage of true positives correctly predicted), and the specificity (percentage of true negatives correctly predicted) (Fielding and Bell 1997). We also calculated Cohen’s Kappa (Titus et al. 1984) from the confusion matrices to assess whether model performance differed from expectations based on chance alone. Cohen’s Kappa ranges from 1 (all cases correctly predicted) to -1 (all cases incorrectly predicted), with a value of zero indicating predictions not different

from random. A Cohen’s Kappa value  $<0.4$  indicate a poor classification performance, 0.4–0.75 is good, and  $>0.75$  is excellent (Landis and Koch 1977).

## Results

### Refuge use

S1 had more fish (16) and a higher density (0.18 fish  $m^{-2}$ ) than S2 (5 fish, 0.04 fish  $m^{-2}$ ) (Fig. 2), but the fish were considerably smaller on S1 ( $14.9 \pm 1.2$  cm, 11–16.3 cm) than on S2 ( $24.0 \pm 3.4$  cm, 20–28 cm). The large fish-size differences between sites and small differences within sites, prevented us from examining size-related effects. During the 10-min focal observations, individual fish occupied 1–9 refuges, with the majority using 1–3 ( $2.7 \pm 2.5$ ). Each *H. rufus* repeatedly visited the same set of refuges but did not use other nearby potential refuges. Fish often moved slowly as they emerged from a refuge, and then swam rapidly to the next refuge. Sometimes, individuals hovered outside but close to a refuge. No refuge was ever used by more than one *H. rufus*. We observed one instance in which a resident *H. rufus* chased a smaller conspecific that attempted to enter its refuge and three cases in which a resident chased a smaller heterospecific from its refuge [one brown chromis, *Chromis multilineata* (Guichenot), one sergeant major, *Abudefduf saxatilis* (Linnaeus), one French grunt, *Haemulon flavolineatum* (Desmarest)]. On three occasions a *H. rufus* shared a refuge with larger or similar-sized heterospecifics [one graysby, *C. cruentata* (Lacépède), one striped burrfish, *Chilomycterus schoepfi* (Walbaum), one spotted moray, *G. moringa* (Cuvier)].

*Holocentrus rufus* most frequently used holes, less often overhangs and occasionally spaces under platforms as refuges (Table 1). The location of refuges on the reef was variable and did not seem highly specific in terms of vertical positioning, distance from the edge or orientation (Table 1). Substrate around the refuge entrance varied between sites, but bare rock was predominant on both (Table 1). Refuges were about equally likely to have open and closed ends (Table 1). The width, height, and length of the refuges varied greatly. Refuge entrance width, height, and area and refuge length differed significantly or nearly significantly between the sites (Table 1).

**Table 1** Characteristics of refuges (mean ± SD, range) unused and used by 21 *H. rufus* at two sites (S1, S2) in the spur and groove zone of Heron Bay Reef, Barbados

Refuge characteristic	Unused		Used by <i>H. rufus</i>		Test statistics ( <i>t</i> or $\chi^2$ ) and <i>P</i> -value			
	S1 ( <i>n</i> = 43)	S2 ( <i>n</i> = 24)	S1 ( <i>n</i> = 39)	S2 ( <i>n</i> = 18)	Unused S1 vs. S2	Used S1 vs. S2	Unused S1 vs. used S1	Unused S2 vs. used S2
1. Height above sand bottom (cm)	57 ± 34 0–133	87 ± 42 13–150	94 ± 28 44–145	71 ± 42 20–150	–2.928 0.099	2.392 0.332	–5.400 <0.001	1.187 >0.95
2. Shortest distance to reef edge (cm)	88 ± 89 0–333	92 ± 82 9–313	158 ± 84 3–303	61 ± 50 8–151	–0.559 >0.95	4.868 <0.001	–4.211 0.002	1.419 >0.95
3. Orientation relative to reef edge (degrees)	73 ± 53 5–180	65 ± 56 0–175	49 ± 50 0–175	58 ± 51 5–160	0.771 >0.95	–0.852 >0.95	2.360 0.311	0.237 >0.95
4. Height above reef substrate (cm)	8 ± 4 4–22	19 ± 10 7–50	14 ± 14 0–73	24 ± 15 6–63	–5.906 <0.001	–2.839 0.126	–2.179 0.384	–0.911 >0.95
5. Vertical relief of reef at entrance (cm)	40 ± 18 17–101	49 ± 17 18–84	53 ± 28 18–120	84 ± 37 35–167	–2.369 0.311	–3.649 0.015	–2.628 0.185	–4.036 0.009
6. Entrance width (cm)	27 ± 18 11–100	41 ± 18 22–94	41 ± 34 10–220	66 ± 34 25–135	–4.146 0.003	–3.248 0.048	–3.023 0.077	–2.822 0.160
7. Entrance height (cm)	16 ± 7 7–40	23 ± 7 12–35	20 ± 13 4–65	37 ± 29 12–125	–3.814 0.009	–3.049 0.078	–1.227 >0.95	–1.692 >0.95
8. Entrance area (cm <sup>2</sup> )	413 ± 458 82–1,963	779 ± 505 225– 2,436	710 ± 831 57–4,084	2,301 ± 2,583 255–8,247	–4.503 0.001	–3.856 0.009	–2.420 0.288	–2.558 0.288
9. Length (cm)	25 ± 12 10–163	32 ± 15 16–71	30 ± 18 7–75	55 ± 28 22–135	–2.191 0.384	–4.268 0.002	–0.936 >0.95	–3.936 0.009
10. Structure type					6.184 0.103	8.247 0.311	10.140 0.119	0.137 0.934
Overhangs (%)	23	38	33	33				
Hole (%)	74	54	59	56				
Space under platform (%)	3	8	8	11				
11. Closure					0.101 0.751	1.048 0.306	2.209 0.137	2.973 0.085
Open end (%)	26	29	41	56				
Closed end (%)	74	71	59	44				
12. Main substrate cover around entrance (%)					27.857 <0.001	14.940 0.011	5.986 0.200	3.422 0.490
Fire coral (%)	14	0	10	6				
Dead coral (%)	2	50	3	33				
Live coral (%)	14	17	33	17				
Bare rock (%)	70	29	54	44				
Sponge (%)	0	4	0	0				

Differences between and within sites were tested with a *t*-test with sequential Bonferroni correction for quantitative refuge characteristics (1–9) and with chi-square for qualitative characteristics (10–12)

*Holocentrus rufus* showed high fidelity to refuges. During daily refuge monitoring, all fish used at least one of the refuges identified during the initial focal observation, and no fish ever occupied a different

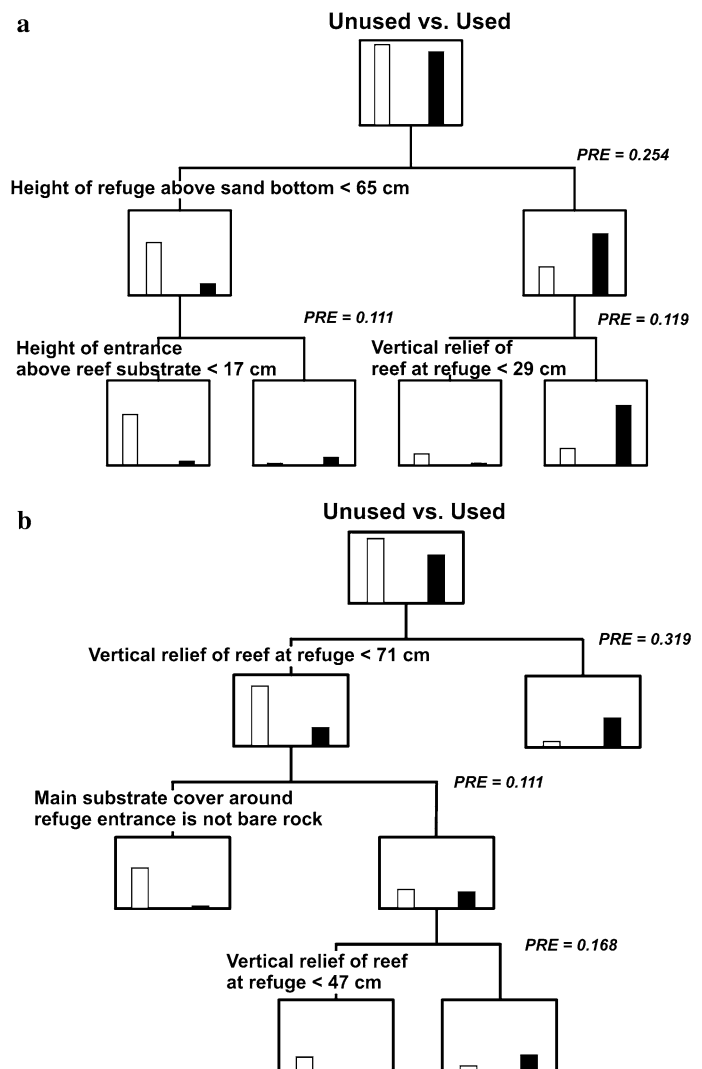
refuge. No fish disappeared during the observation periods, and there was no immigration to the study sites between the tagging of the first fish and the final observation (28 days).

## Refuge selection

Although used refuges were highly variable, there was evidence that *H. rufus* showed selection. Used refuges differed from unused potential refuges in height above the surrounding sand bottom and distance to the reef edge on S1 and in vertical relief of the reef at the entrance and in refuge length on S2. Used refuges were higher above the sand and farther from the edge than unused potential refuges on S1, but the trend was opposite on S2. In all other characteristics except orientation, there was a trend for used refuges to have higher values than unused potential refuges on both S1 and S2 (Table 1).

The CT models explained a high proportion of the variation (S1: PRE = 0.484; S2: PRE = 0.598). On S1 (Fig. 3a), the characteristics that best predicted whether a refuge was used were height above sand greater than 65 cm (PRE = 0.254) and a vertical relief of the reef at the refuge greater than 29 cm (PRE = 0.119). If the height of refuge above the sand was less than 65 cm, used refuges had a height of entrance above reef substratum greater than 17 cm (PRE = 0.111). On S2 (Fig. 3b), the characteristic best predicting refuge use was vertical relief of the reef at the refuge greater than 71 cm (PRE = 0.319). When the vertical relief was smaller than 71 cm, the occupied refuges were characterized by bare rock

**Fig. 3** (a) Classification tree model for predicting the potential refuges that were unused ( $n = 43$ ) versus the refuges used ( $n = 39$ ) by *H. rufus* ( $n = 16$ ) at S1 and (b) classification tree model for predicting the potential refuges that were unused ( $n = 24$ ) versus refuges used ( $n = 18$ ) by *H. rufus* ( $n = 5$ ) at S2. Vertical bars represent the frequency of potential refuges that were unused (white) and the used refuges (black). Splitting values and proportional reduction in error (PRE) are specified on each branch of the tree





substratum around the entrance (PRE = 0.111) and a vertical relief of the reef at the refuge higher than 47 cm (PRE = 0.168).

Both CT models were very accurate in predicted used and unused refuges using the data from which the model was built (calibration), with CCRs close to 85% (Table 2). The models more accurately predicted refuges used (calibration sensitivity) than refuges not used (calibration specificity) on both sites (Table 2). When we applied the models for each site to data from the other (validation), accuracy dropped considerably as indicated by lower CCR, especially for the model from S2 (Table 2). Model sensitivity also showed major reductions, but specificity declined only on S2. As a measure of model performance, Cohen’s Kappa (Table 2) was high in calibration with values near 0.7 but much lower in validation, with negative values in S2.

**Discussion**

*Holocentrus rufus* did not specialize on a narrow set of refuge characteristics. They used holes and overhangs with a wide range of shapes, sizes and positions on the reef. Shulman (1985) observed this species using the cavities of conch shells. Despite the broad range of refuges accepted, there was evidence for selectivity. First, the comparison of individual characteristics showed that used refuges differed from potential but unused refuges in several measures on both reefs. Second, the CT models differentiated used from unused potential refuges on both reefs. These models explained much of the variation and predicted refuge use with high accuracy. On both

reefs, the characteristics that best differentiated used from unused refuges were measures of vertical relief, height of the entrance above the adjacent reef, and height above the surrounding sand, indicating that *H. rufus* preferred refuges situated higher on steep surfaces of these reefs. Holes in these positions may facilitate the detection and avoidance of larger transient predators such as snappers, jacks and barracudas.

The poor transferability between sites does not contradict the high predictability of refuge use on each site. Differences between sites in habitat selection as a result of differences in refuge availability, fish size, and local density may explain the poor transferability of the models. In addition, the use of larger refuges by the larger fish on S2 suggests that additional studies over a broader range of locations may find relationships between fish size or population density on refuge size.

We have not found other studies that measured the characteristics of the diurnal refuges of nocturnal reef fishes with which to compare our results. Indeed, there are few quantitative measures of refuge characteristics for any reef fish. For other coral reef species, size has been the most frequently reported refuge measure. Hixon and Beets (1989, 1993) showed experimentally that the size of several species of coral reef fish, including holocentrids, was related to the size of the holes provided on artificial reefs constructed of concrete blocks. For bridled gobies, *Coryphopterus glaucofraenum* (Gill), Forrester and Steele (2004) reported that the crevices used as refuges when threatened were located where solid objects bordered sand patches and ranged from 3–55 cm wide and 2–16 cm high with a depth of

**Table 2** Comparison of three different measures to evaluate classification tree model accuracy [correct classification rate (CCR), specificity, sensitivity] and one measure to evaluate model performance (Cohen’s Kappa coefficient)

Measures to evaluate model accuracy and performance	S1		S2	
	Calibration (S1–S1)	Validation (S1–S2)	Calibration (S2–S2)	Validation (S2–S1)
Correct classification rate (CCR) (%)	84.15	62.2	85.71	35.71
Sensitivity (%)	92.31	46.15	88.88	66.66
Specificity (%)	76.74	76.74	83.33	12.5
Cohen’s Kappa coefficient	0.685	0.232	0.712	-0.189

Measures are given for used and unused refuges for *H. rufus* in sites S1 and S2

3–50 cm. Spinyhead blennies, *Acanthemblemaria spinosa* Metzelaar, occupy empty worm holes in coral that are deep enough for the whole body and slightly larger in diameter than the fish with the size of occupied holes increasing with body size (Buchheim and Hixon 1992; Clarke and Tyler 2003). The nocturnal shelters of the diurnal bluehead wrasse, *Thalassoma bifasciatum* (Bloch), were also often tubular, formed a tight fit to the body, and hid the fish from outside view, though exceptions to this pattern were quite frequent (Robertson and Sheldon 1979). In contrast, the sleeping sites of the damselfishes *Stegastes dorsopunicans* (Poey) and *S. planifrons* (Cuvier) tended to be considerably larger than the fish and more open to view from the outside (Robertson and Sheldon 1979). Among the few studies examining other characteristics, Clarke (1994) and Clarke and Tyler (2003) showed that holes used by chaenopsid blennies differed between species and sexes in substrate type, hole orientation, height above the main reef surface. Clarke (1994) provided experimental evidence for a preference for hole orientation in *A. spinosa*.

On Heron Bay Reef, *H. rufus* often used multiple refuges, sometimes hovering near the entrance, and moving so frequently among them that all used shelters could be identified in less than 10 min. This pattern appears to differ from the behavior observed by Collette and Talbot (1972) who observed *H. rufus* outside holes only at night. Shulman (1985) also reported that individuals occupying conch shells remained so deep within the cavity that they were barely visible. The simultaneous use of multiple holes does not appear to have been described previously for refuge-using species. Various species, including moray eels and bluehead wrasses, have been reported to use multiple refuges, but they switch between them on a time scale of days rather than minutes (Robertson and Sheldon 1979; Young and Winn 2003).

*Holocentrus rufus* showed strong site fidelity to both reefs and refuges, with no individuals entering or leaving the study population or adding additional refuges during more than 20 days of monitoring. Winn et al. (1964) and Shulman (1985) reported similar fidelity in *H. rufus*. Strong fidelity to refuge holes has been reported in several other nocturnal reef fish species (Marnane 2000; Meyer et al. 2000; Annese and Kingsford 2005).

Unlike many other nocturnal species that school in their diurnal shelters, *H. rufus* appeared never to share refuge holes with conspecifics. Because refuges used by different individuals were sometimes close enough to have easily allowed spatial overlap (Fig. 2), the lack of refuge sharing may indicate a territorial system, as concluded by Winn et al. (1964). Although we observed only a single case of intra-specific aggression, this may have been due to a lack of intrusion. Intraspecific chasing, displays, and acoustical threats were described by Winn et al. (1964). Preliminary studies in which we simulated an intrusion by placing a *H. rufus* in a plastic bag near the refuge of another individual sometimes resulted in an aggressive response by the resident (D. L. Kramer, unpublished observations). In addition, *H. rufus* defended refuges against various smaller heterospecifics. Similar observations were made by Shulman (1985). The tolerance of larger heterospecifics that we observed may have been due to the difficulty of defending against larger fishes. Two of these heterospecifics are known predators of *H. rufus*, but they were definitely too small to feed on the two associated individual *H. rufus*. Many other species defend refuges (e.g., Robertson and Sheldon 1979; Shulman 1985; Buchheim and Hixon 1992; Clarke and Tyler 2003). Although several studies suggest that refuge holes can be a limiting resource (Shulman 1984; Hixon and Beets 1989, 1993; Buchheim and Hixon 1992; Caley and St-John 1996; Clarke and Tyler 2003; Forrester and Steele 2004) other studies provide evidence for a surplus of holes (Robertson and Sheldon 1979; Robertson et al. 1981). It is not clear why the fish defended multiple refuges. Shulman (1985) suggested that defending multiple refuges provides an alternative location if a fish is displaced by a larger conspecific or needs to flee a predation attempt. Because *H. rufus* forages on reefs (Gladfelter and Johnson 1983), the area near their refuges may be important feeding sites, so the fish may be defending part of their nocturnal foraging area rather than defending refuges per se.

In conclusion, this study provides the first description of the diurnal refuges of a nocturnal reef fish and the first evidence of selectivity for such sites. Combined with the use of multiple refuges by some individuals and exclusive use, probably due to defense, this suggests that interference competition for diurnal refuges could affect the distribution and

abundance of *H. rufus* on natural reefs. Further studies of refuge preference and refuge availability in this and related species are currently in progress.

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