Validation of a randomization procedure to assess animal habitat preferences: microhabitat use of tiger sharks in a seagrass ecosystem

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Summary

1. Tiger sharks *Galeocerdo cuvier* are important predators in a variety of nearshore communities, including the seagrass ecosystem of Shark Bay, Western Australia. Because tiger sharks are known to influence spatial distributions of multiple prey species, it is important to understand how they use habitats at a variety of spatial scales. We used a combination of catch rates and acoustic tracking to determine tiger shark microhabitat use in Shark Bay.

2. Comparing habitat-use data from tracking against the null hypothesis of no habitat preference is hindered in Shark Bay, as elsewhere, by the difficulty of defining expected habitat use given random movement. We used randomization procedures to generate expected habitat use in the absence of habitat preference and expected habitat use differences among groups (e.g. males and females). We tested the performance of these protocols using simulated data sets with known habitat preferences.

3. The technique correctly classified sets of simulated tracks as displaying a preference or not and was a conservative test for differences in habitat preferences between subgroups of tracks (e.g. males vs. females).

4. Sharks preferred shallow habitats over deep ones, and preferred shallow edge microhabitats over shallow interior ones. The use of shallow edges likely increases encounter rates with potential prey and may have profound consequences for the dynamics of Shark Bay's seagrass ecosystem through indirect effects transmitted by grazers that are common prey of tiger sharks.

5. Females showed a greater tendency to use shallow edge microhabitats than did males; this pattern was not detected by traditional analysis techniques.

6. The randomization procedures presented here are applicable to many field studies that use tracking by allowing researchers both to determine overall habitat preferences and to identify differences in habitat use between groups within their sample.

Key-words: foraging, habitat availability, habitat use, predator–prey interactions, tracking.

Introduction

Patterns of animal habitat use influence the structure and dynamics of communities because they shape the nature and probabilities of interspecific interactions (e.g. Morris 2003). Habitat use by top predators is especially important because these species have the potential to influence the distribution and abundance of prey and other species both directly and indirectly (e.g. Lima & Dill 1990; Estes *et al*. 1998; Werner & Peacor 2003; Nelson, Matthews & Rosenheim 2004; Schmitz, Kriva & Ovadia 2004). Despite the importance of such data, studies of habitat use by large-bodied taxa,

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including many top predators, are underrepresented in the literature, largely because these animals are often elusive and range widely. With the development of a variety of tracking techniques (e.g. acoustic, radio and satellite telemetry), however, obstacles facing studies of the movements of large predators have been greatly reduced.

In Shark Bay, Western Australia, tiger sharks *Galeocerdo cuvier* are a top predator with the potential to influence a variety of species within the local seagrass ecosystem through direct and indirect effects (e.g. Heithaus 2001; Heithaus & Dill 2002; Heithaus *et al.* 2002a; Heithaus, Frid & Dill 2002b; Dill, Heithaus & Walters 2003). Previous studies have shown that tiger sharks prefer shallow habitats to deep ones (Heithaus *et al.* 2002a). However, there is microhabitat variation in prey abundance, prey vulnerability, current velocity and other variables within these larger habitat types. Determining how sharks use these microhabitats will help elucidate habitat-use decisions made by sharks and their prey. Measuring microhabitat preferences in Shark Bay is difficult because: (1) tiger sharks have extremely large (or no defined) home ranges (Heithaus 2001); (2) tiger sharks show directional biases irrespective of habitat type (Heithaus *et al.* 2002a); and (3) the habitat exhibits a patchy structure and tracks are not easily started in a random fashion with respect to these patches (Heithaus *et al.* 2002a). Therefore, it is important to use an appropriate null hypothesis (i.e. expectation of observed habitat use when there is no habitat preference) when drawing conclusions about microhabitat preferences from tracking data.

A common goal of tracking studies, including ours, is to determine whether the habitats used during a track reflect random movements or an underlying habitat preference. Another goal is to determine whether individuals that differ in some way (e.g. age, sex, etc.) use habitats differently. It is often difficult to conduct quantitative analyses of habitat-use data obtained from tracking because it may be unclear what the expected habitat use would be given random movement. Often, the entire study area is considered to be available to each individual and the average proportion of each habitat in the study area or within an animal's home range is used as the measure of availability of that habitat for each animal (e.g. Clark, Leslie & Carter 1993; Manly, McDonald & Thomas 1993). This approach is likely to be valid in studies where animals have well-defined home ranges and a large number of position samples, both in space and time, are obtained (e.g. Morrissey & Gruber 1993). However, the expectation that randomly moving animals should use habitats in proportion to the environmental average is often not valid and may lead to inaccurate conclusions about habitat preferences, or lack thereof (e.g. Porter & Church 1987; Manly *et al.* 1993; Arthur *et al.* 1996; Wilson, Shackleton & Campbell 1998). First, the spatial scale at which animals make habitat-use decisions may have little to do with the spatial scale of the study area. Also, each habitat-use

observation during a track is constrained by the starting position and all previous moves, unless there is a long period between observations. This alters the spatial scale at which habitat-use questions can be addressed. In addition, if starting positions of tracks are not random with respect to habitat, expected habitat use, even with random movements, would not be the same as average availability of that habitat in the study area. Finally, expected habitat use under random movement is difficult to assess if habitats are irregularly distributed and patchy, animals are tracked over relatively short periods, have ill-defined home ranges, or show directional biases to their movements that are unrelated to habitat preference.

We developed randomization techniques for comparing observed tracking data with the null hypothesis of random use to investigate microhabitat preferences of tiger sharks in Shark Bay. We illustrate the applicability of our techniques by presenting analyses of computer-simulated tracks with known preferences, and suggest that they can be employed in many field situations including those where sample sizes are relatively small and observations can only be made over relatively short time periods.

Materials and methods

STUDY SITE

The study was conducted in the Eastern Gulf of Shark Bay, a large semi-enclosed bay along the central coast of Western Australia (25°45′S, 113°44′E; Heithaus 2001). The bay contains a large populations of tiger sharks and many potential prey species including green *Chelonia mydas* and loggerhead turtles *Caretta caretta*, dugongs *Dugong dugon*, sea snakes, seabirds, teleosts, and bottlenose dolphins *Tursiops aduncus* (e.g. Heithaus 2001; Heithaus *et al.* 2005). Within the study site there is considerable habitat patchiness. Generally, habitats can be divided into shallow banks (≤ 4·5 m deep), which are largely covered by seagrass, and surrounding deeper waters (generally 6–12 m) with primarily sandy bottoms. These can be subdivided into four microhabitats. From the standpoint of predators and their prey, shallow banks can be divided into interior and edge habitats. Because of tiger sharks' limited manoeuvrability relative to many of their prey (Heithaus *et al.* 2002a), capturing prey in interior habitats is likely to be easier as the water tends to be under 2·5 m and there are few directions in which prey can escape. In edge habitats, prey may be less vulnerable because they can more easily escape to nearby deeper waters. Furthermore, the water is usually 2·5–4·5 m deep in this microhabitat, and prey may be better able to manoeuvre. Because of these differences in vulnerability, as well as possible depth-related differences in the quality of seagrass available to herbivores (unpublished data), it is likely that these shallow microhabitats also differ in the availability of prey. Deep habitats can be subdivided into open expanses of deep water, with relatively low

current velocities, and channels, where currents can exceed 3 km h^{-1}. In this study we define these four microhabitats as follows: deep channel, deep open water, shallow interior (< 2·5 m depth and > 75 m from water > 4·5 m depth), and shallow edge (waters < 2·5 m depth within 75 m of water > 4·5 m depth, and waters 2·5–4·5 m depth).

FIELD METHODS

From 1997 to 2004, sharks were captured using drumlines equipped with a single hook (Mustad Shark Hook size 12/0, 13/0, or 14/0) baited primarily with commercially available Australian salmon *Arripis truttaceus* and occasionally with other teleosts (see Heithaus 2001 for details). Lines were spaced approximately 700 m apart and were often set in multiple habitats or microhabitats concurrently. Lines were checked every 2–4 h and bait presence/absence was noted.

From 1997 to 2000, two methods were used to track sharks (see Heithaus *et al.* 2002a). For standard acoustic tracking, sharks (*n* = 8) were fitted with an acoustic transmitter (V32, 28·5–36·0 kHz, VEMCO, Shad Bay, Nova Scotia) inserted into the peritoneal cavity (see Holland *et al.* 1999:; Heithaus *et al.* 2002a; for details). Alternatively, an animal-borne video and environmental data collection system (AVED, National Geographic's 'Crittercam'; Heithaus *et al.* 2001) equipped with an acoustic transmitter was positioned on the dorsal fin (*n* = 37; see Heithaus *et al.* 2001). AVED were left attached to sharks for up to 11 h, and standard tracks were made for up to 18 h.

Sharks were tracked from a 4·5-m research vessel using a directional hydrophone (V11, VEMCO or DH-4, Sonotronics, Tucson, AZ) and an acoustic receiver (VR-60, VEMCO or USR-5W, Sonotronics). Every 5 min we recorded the boat GPS location ('position'), direction to the shark, estimated distance to the shark (based on signal strength), and shark's habitat. In general, when sharks remained in the same microhabitat and travelled in a linear fashion, we maintained a distance of approximately 200 m from the shark and the position of the boat relative to the shark was changed frequently. If the habitat at the estimated position of the shark was possibly different from that of the boat, the boat was moved to the shark's estimated position after it had moved an estimated 100 m away in order to avoid boat disturbance to the shark. Using this technique, the GPS positions recorded on the boat were different from that of the shark at any particular moment, but the recorded boat positions relative to the shark's actual path were accurate at the scale of tens of metres. This error is far smaller than the scale of microhabitat patches, which are usually hundreds to over 1000 m wide and over 1000 m long. Because the real-time position of the shark is not critical to microhabitat-use questions examined in this study, time lags between the shark passing a point and the boat obtaining a position fix do not compromise analyses. Furthermore, estimated position errors provided by GPS units in Shark Bay are

generally under 10 m (unpublished data), and microhabitat designations recorded in the field were compared with those provided in the GIS to check for large errors in position.

AVED systems disengaged from sharks automatically and floated to the surface where they were retrieved using VHF telemetry (MOD-050, 150·0–151·0 MHz transmitter; TR-4 receiver Telonics, Mesa, AZ). Position estimates and the habitat a shark was using at each position were corrected using video and depth data (Heithaus *et al.* 2001). Video data obtained from AVED, long-term tracking data of tiger sharks, and observations of free-swimming animals indicate that tiger shark behaviour is not impacted substantially by capture and instrumentation (Heithaus *et al.* 2001, 2002a). For example, sharks were observed foraging and chasing potential prey items within several minutes of release and exhibited swimming patterns (e.g. vertical movements, displacement rates) in the first 30 min of tracks identical to those after more than 12 h (Heithaus *et al.* 2002a). Furthermore, there was no difference in microhabitat use of sharks that experienced minimal handling times and were tracked by AVED compared with those with surgically implanted transmitters ($P = 0·16$, $P = 0·45$, $P = 0·28$, $P = 0·10$ for open, channel, edge and interior microhabitats, respectively, based on the identity randomization technique before correction for multiple comparisons; see below) suggesting that handling time did not influence microhabitat preferences of sharks. Similarly, there was no difference in microhabitat use of sharks that were tracked for greater than 3 h vs. less than 3 h ($P = 0·39$, $P = 0·12$, $P = 0·39$, $P = 0·27$ for open, channel, edge and interior microhabitats, respectively, based on identity randomization technique before correction for multiple comparisons; see below).

Because of rapid loss of baits to species other than tiger sharks in shallow habitats, it is not possible to use catch rates to examine habitat use or microhabitat use within shallow habitats (Heithaus 2001). However, we used differences in catch rates within deep habitats to test for differences related to microhabitat (deep channel vs. deep open waters) and water depth for comparisons with data obtained from tracking. For these analyses, we assumed that bait was lost halfway through the fishing period for hooks that had either lost bait or caught a shark (Heithaus 2001). We calculated catch rates in each microhabitat for each fishing day, and compared these among microhabitats while taking monthly and annual variation into account using ANOVA on log $(x + 1)$ transformed data. Differences in shark catches among four depth classes of deep habitats (4·5–6·5 m, 6·5–8·5 m, 8·5–10·5 m, 10·5–12·5 m) were investigated using chi square with expected catches determined by fishing effort in each depth range.

RANDOMIZATION PROTOCOL

We developed track randomization techniques (see Appendix I for validation tests of these techniques) to

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test for microhabitat preferences of tiger sharks in Shark Bay and to determine whether habitat and microhabitat preferences varied with size or sex. We use the following definitions. *Positions* are the points at which an individual's location and current habitat are determined. *Habitat use* is defined as the proportion of positions that occur within a particular habitat. A *step* consists of the direction and distance travelled between positions. A *track* is the set of steps made by an individual. *Habitat preference* refers to the use of a habitat significantly more often than expected based on movements that are random with respect to habitat type and *habitat avoidance* indicates the use of a habitat significantly less often than expected based on random movements.

Randomization techniques are based on the assumption that, if a set of observations were drawn from a random distribution, then all possible orders of those observations would be equally likely to have occurred. Therefore, a test statistic describing the set of observations should not differ significantly from a distribution of that test statistic generated by randomly reordering the observations (Manly 1997). Our method to determine overall microhabitat preference and avoidance compares the observed microhabitat use of the sample (with each individual contributing a single microhabitat-use value) with microhabitat-use values determined from tracks that have been randomly reallocated among starting positions with the directions, distances and sequences of steps in each track retained ('track reassignment' protocol; see Appendix I). To accomplish randomizations using field data, the 45 tiger shark tracks were each randomly reassigned among the observed starting locations (all starting locations were used for each randomization iteration). The resulting tracks then were mapped into a GIS (MapInfo, MapInfo Corp.) of the study area (generated using existing nautical charts that were ground-truthed) and the microhabitat associated with each position appended to the track. We then determined the average use of each microhabitat for the randomized sample of tracks, with each track contributing one set of habitat-use proportions to the overall mean. Observed microhabitat use of tiger sharks was compared with the distribution of mean microhabitat-use values obtained from 500 randomization iterations. We considered sharks to exhibit a significant microhabitat preference or avoidance if less than 12 of 500 randomizations (2·5%) produced simulated microhabitat-use values that were greater than, or less than, the observed use of that microhabitat (i.e. $P = 0·05$ for a two-tailed test). We tested whether sharks preferred one type of deep or shallow microhabitat over the other by comparing values of mean observed use of (1) shallow interior minus shallow edge, and (2) deep channel minus deep open to values for these differences obtained from randomizations. To correct for multiple comparisons, we applied a Bonferonni correction. Therefore, we considered results to be significant at $P < 0·05$ if less than 4 of 500 (0·83%) randomly generated habitat-use values or differences were either greater than or less than observed values. To avoid confusion, we report two-tailed P-values before correction.

We used two randomization techniques to determine whether subgroups [i.e. males vs. females and > 325 cm total length (TL) vs. < 325 cm TL] of our tiger shark sample differed in habitat and microhabitat preference. In the first, we reassigned tracks among the observed starting positions as above ('track reassignment') and then compared the observed difference in mean use of a microhabitat between subgroups (e.g. mean male use of edge microhabitats minus mean female use of edge microhabitats) against the distribution of differences in mean use of that microhabitat generated through randomizations. For the 'identity randomization' protocol, we randomly reassigned a subgroup identity (e.g. male or female) to each track, keeping the ratio of the subgroups within the randomized sample identical to that of the original sample. Tracks were not shuffled among starting positions. For each randomization iteration, we recorded the difference in use of each microhabitat between groups. We considered results to be significant if the observed habitat or microhabitat-use differences between sexes or size classes was greater than or less than 12 of 500 or 50 of 2000 (2·5%) of the differences obtained through track reassignment and identity reassignment procedures, respectively.

Results

Tracks of tiger sharks ($n = 37$ AVED; $n = 8$ standard acoustic) revealed a preference for both shallow microhabitats over deep ones, but this was significant only for shallow edge microhabitats (Fig. 1). Randomly generated values for shallow interior microhabitat use were greater than the observed use in 21 of 500 iterations ($P = 0·08$), while none of the randomly generated values for shallow edge microhabitat use were greater than was observed during tracks ($P < 0·002$). Also, sharks preferred edge microhabitats over interior ones. None of 500 randomization iterations resulted in greater mean use of edge microhabitats relative to interior ones than was observed ($P < 0·002$).

There was neither preference for nor avoidance of deep open microhabitats; 35 of 500 randomly generated values of microhabitat use were lower than the observed use ($P = 0·14$). Sharks appear to avoid deep channels, but the result is not significant after correction for multiple comparisons. Randomly generated values of channel microhabitat use were lower than the observed use in seven of 500 iterations ($P = 0·028$). Sharks tended to use deep open waters more often than channels, but the difference was not significant for tracked individuals. Observed mean relative use of deep open microhabitats relative to channels was greater than that obtained from all but 27 of 500 randomization iterations ($P = 0·11$).

Microhabitat differences in catch rates generally agreed with tracking results. However, catch rates were higher in deep open microhabitats ($\bar{x} \pm$ SD = 0·08 ± 0·13 sharks* h^{-1}, $n = 144$ captures) than channels ($\bar{x} \pm$ SD = 0·06 ±
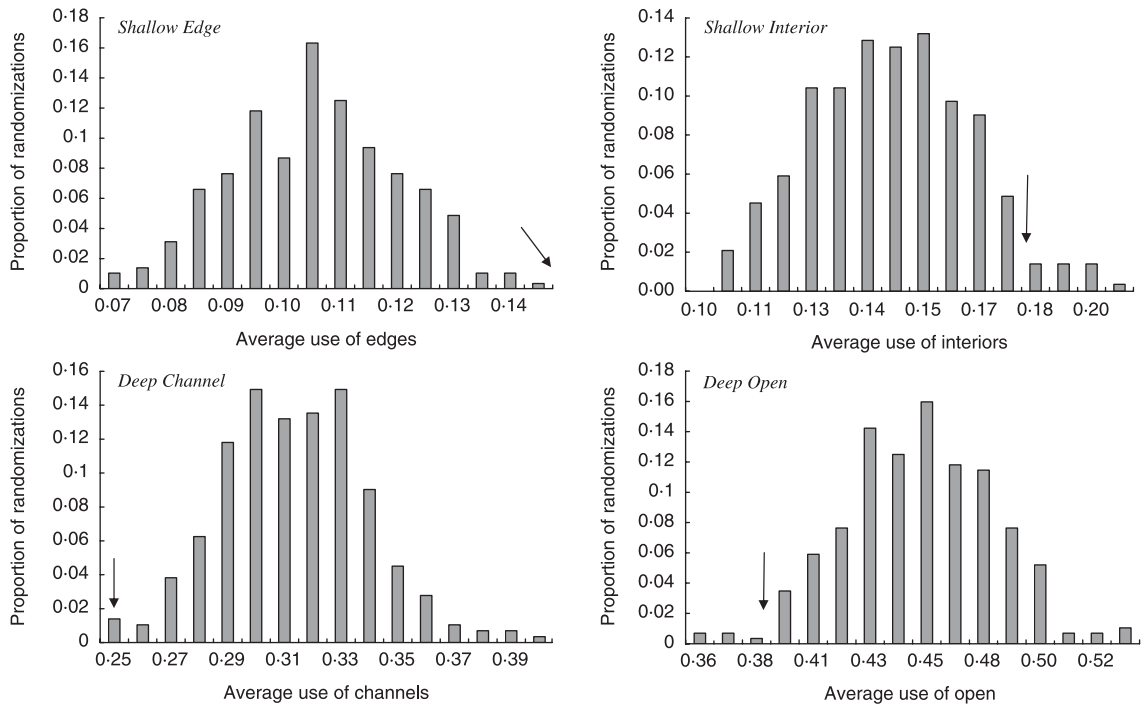
Fig. 1. Expected distribution of the proportion of shark positions in each microhabitat after randomly re-assorting tracks among starting positions 500 times. Arrows represent observed values.
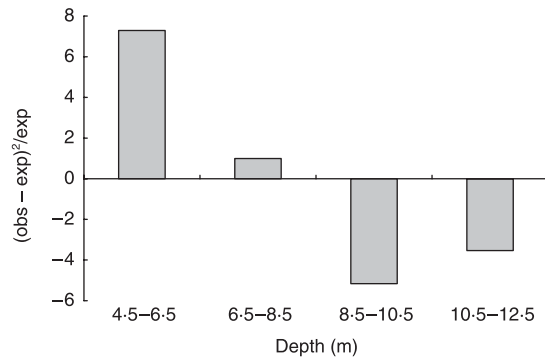

Fig. 2. Catches of tiger sharks relative to expectations based on sample effort in four depth classes of deep-water habitats. Bars with negative numbers reflect fewer sharks captured than expected.

0.06 sharks* h^{-1}, $n = 163$ captures; $F_{1,306} = 4.10$, $P = 0.044$). Also, catch rates were higher than expected in the shallowest depth ranges of deep habitats ($\chi^2 = 17.0$, d.f. = 3, $P < 0.001$, $n = 386$ captures; Fig. 2). Results were unchanged if only sets using Australian salmon for bait were included ($\chi^2 = 17.0$, d.f. = 3, $P < 0.001$, $n = 258$ captures).

Sex differences in habitat and microhabitat use were detected using both track reassignment and identity reassignment protocols. Although both male and female sharks showed a preference for shallow habitats, the magnitude of this preference was greater in females. Females ($n = 27$) were observed to use shallow habitats 16% more often than males ($n = 18$). Track reassignment randomizations produced only seven of 500 values that exceeded this difference between sexes ($P = 0.028$) and

identity (sex) reassignment produced 28 of 2000 values ($P = 0.028$) greater than 16%. The sex difference in habitat use was primarily driven by female sharks' relatively greater use of edge microhabitats (despite both sexes preferring this microhabitat over the other three) and relatively lower use of deep open microhabitats than males (Fig. 3). None of 500 track reassignment (TR) randomization iterations and only 13 of 2000 identity reassignment (IR) randomization iterations resulted in greater values of female use of edge microhabitats relative to males compared with the observed sex difference in use of this microhabitat ($P < 0.002$, $P = 0.0065$, respectively). Only 12 of 500 ($P = 0.048$) and 83 of 2000 ($P = 0.083$) randomizations produced greater differences in the use of deep open habitats by males vs. females than was observed for track reassignment and identity reassignment, respectively. There were no apparent sex differences in use of shallow interior [$P = 0.64$ (TR); $P = 0.18$ (IR)) and deep channel ($P = 0.78$ (TR); $P = 0.47$ (IR)] microhabitats.

There were no differences in habitat or microhabitat use by sharks longer than 325 cm TL compared with those shorter than this. The observed difference between the size classes in their use of shallow and deep habitats was less than that of 249 of 500 values ($P = 0.99$) and 245 of 500 values ($P = 0.98$) generated by track rearrangement. Results were the same for identity reassignment ($P = 0.49$ for shallow and $P = 0.48$ for deep habitats). Observed differences between size classes were greater than those obtained for 32, 299, 223 and 410 of 500 randomly generated differences using track reassignment for channel ($P = 0.13$), edge ($P = 0.80$), interior ($P = 0.90$) and open ($P = 0.36$) microhabitats, respectively. Results
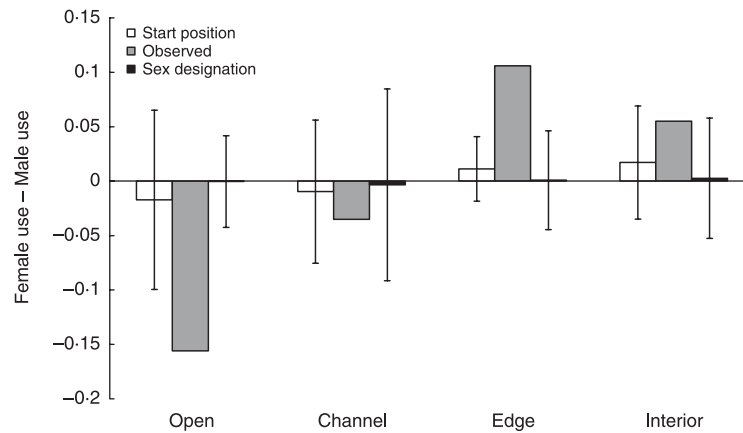
Fig. 3. Sex differences in tiger shark habitat use. Bars represent average female use of a microhabitat minus average male use of a microhabitat. Bars rising above the axis represent relatively greater female use of a microhabitat while those falling below the axis represent greater male use of a microhabitat. Randomizations revealed greater use of shallow edge habitats by females and greater use of open deep waters by males regardless of the randomization protocol that was used. Note that both procedures are useful in discriminating magnitude of habitat preference since both males and females prefer edge microhabitats. Error bars are ± SD.

based on identity reassignment were similar with the observed difference between size classes greater than the randomly generated values of 1087, 1673, 858 and 1485 of 2000 iterations for channel ($P = 0.91$), edge ($P = 0.33$), interior ($P = 0.86$) and open ($P = 0.52$) microhabitats, respectively.

Discussion

TIGER SHARK MICROHABITAT USE

We found that tiger sharks prefer shallow habitats over deep ones (Heithaus *et al*. 2002a). However, sharks exhibited preferences within shallows at the microhabitat level. Sharks showed a strong preference for shallow edge microhabitats over shallow interior ones. Tracked sharks did not show a significant preference for one deep microhabitat over the other. Catch rates, however, were higher in deep open waters than deep channels, with two more sharks caught in deep open waters per 100 fishing hours. Also, shark catches were highest in shallow parts of deep habitats, which may be due to capture of sharks from edges that had moved into deeper waters to take baits. Although these sharks must move constantly and have extremely large (or no defined) home ranges, our results show that they still select habitats at several spatial scales, including habitat patches (kilometres) and microhabitats (hundreds of metres).

A previous analysis of tracking data used in this study detected no sex differences in the proportion of time spent in deep and shallow habitats for Shark Bay tiger sharks (Heithaus *et al*. 2002a). However, our randomization procedure revealed that female sharks used shallow areas, especially shallow edges, more often than males even though both sexes preferred shallow habitats over deep ones. These differences were found even with the conservative track randomization technique, which suggests strongly that this preference is real. It is

somewhat surprising that the randomization technique revealed significant differences between the sexes in habitat and microhabitat preference as the two sexes were captured in the same habitats during the same time period. However, differences in the average starting position of males and females with respect to habitat patches leads to a null hypothesis of females using shallow habitat less than males if there were no differences in habitat preference. Thus, the null hypothesis of the previous study, that males and females should use habitats in identical ratios, was incorrect despite relatively large sample sizes and haphazard starting locations of tracks. That this problem could arise with relatively large samples sizes (especially for large marine predators such as sharks) highlights the importance of using appropriate measures of expected habitat use.

Sex differences in habitat use, and even habitat segregation by sex, are widespread in sharks. For example, lesser spotted dogfish *Scyliorhinus canicula* off south-west Ireland appear to segregate into deep and shallow-water habitats, with males spending days resting in deep waters and foraging in shallows at night and females spending days inside shallow-water caves and venturing into deep waters at night to feed. This pattern of segregation appears to be a result of female avoidance of male harassment associated with reproduction (Sims, Nash & Morritt 2001). Although unrelated to reproduction, there appears to be sexual segregation of small tiger sharks in Shark Bay. Sex ratios of animals < 3 m are extremely female-biased, but are approximately 1 : 1 for sharks > 3 m (Heithaus 2001). The current study shows sex differences in habitat use of large sharks at a fine spatial scale, even though there is not segregation of males and females. The reason for the difference is unclear.

Tiger shark preference for shallow habitats is likely due to the high prey densities found in these areas, relative to deeper waters. Dugongs *Dugong dugon*, sea turtles *Caretta caretta* and *Chelonia mydas*, sea snakes

primarily *Hydrophis elegans*, pied cormorants *Phalacrocorax varius* and teleost fishes are all found in higher abundance in shallow habitats (Heithaus *et al.* 2002a; Heithaus 2004a, 2005). Both prey density and probability of prey capture appear to vary with microhabitat. The probability of prey capture should be higher in shallow interior habitats than in shallow edge habitats, primarily due to the limited escape routes available to prey in the former (Heithaus *et al.* 2002a,b; Heithaus 2004b). Therefore, tiger sharks might be expected to favour shallow interior habitats if prey abundance were equal across the shallows. The abundance of primary prey species, however, appears to be greater in edge habitats (e.g. Wirsing 2005; Heithaus & Dill 2006). Thus, tiger sharks may maximize encounter rates by swimming through these shallow edge habitats rather than across the middle of banks, and their preference for shallow edge microhabitats over shallow interior ones may reflect a response to prey abundance at a restricted spatial scale. However, sharks still spend considerable time within shallow interior habitats relative to predicted use under the null hypothesis of no preference, possibly sampling this microhabitat for prey that should be easier to catch.

Sharks may also select edge habitats to minimize energy costs or to improve their ability to detect olfactory stimuli from both shallow and deep waters. To conserve energy, sharks should select swift-moving waters when flow is in the direction of travel and move out of them when flow is opposite of travel direction. Sharks show an underlying trend to move generally north regardless of habitat and there are no differences in habitat-use patterns based on tidal direction (Heithaus *et al.* 2002a), which is inconsistent with the energetic saving hypothesis. The olfactory detection hypothesis remains untested.

The risk of predation posed by tiger sharks influences habitat-use decisions by multiple prey species in Shark Bay (e.g. Heithaus & Dill 2002; Dill *et al.* 2003; Heithaus 2005; Wirsing 2005). For example, bottlenose dolphins *Tursiops aduncus* largely abandon shallow habitats that are rich in prey when tiger shark abundance is high (Heithaus & Dill 2002), and both pied cormorants and dugongs reduce their use of dangerous shallow habitats, where sharks are most abundant, with increasing tiger shark density (Heithaus 2005; Wirsing 2005). Our finding that tiger sharks select habitats at the microhabitat level raises the possibility of habitat selection games between predators and prey in Shark Bay at a restricted geographical scale. Such fine-scale habitat-use decisions could have profound consequences for the Shark Bay ecosystem through behaviourally mediated indirect species interactions (BMII). Because tiger sharks feed on dugongs and green sea turtles, which may have a substantial impact on the structure of seagrass communities (e.g. Lanyon, Limpus & Marsh 1989; Bjorndal 1997; Aragones & Marsh 2000), microhabitat shifts in these species in response to tiger sharks could alter the structure and dynamics of the community (Walker 1989). BMII between predators and plants have been identified as important in structuring terrestrial communities (see Schmitz, Beckerman & Brien 1997; Schmitz *et al.* 2004). For example, the presence of wolves has a profound influence on plant community structure through cascading consequences of habitat shifts by herbivores (e.g. Beschta 2003; Ripple & Beschta 2003, 2004; Fortin *et al.* 2005). We suggest therefore that large marine predators, such as tiger sharks, may play roles similar to those of wolves and other large predators in terrestrial systems.

RANDOMIZATION PROTOCOL

The techniques that we have presented here are useful tools for assessing animal habitat preferences from tracking data when expected habitat use under random movements is unclear, track starting positions are nonrandom, or animals can only be observed over relatively short time frames, and provides advantages over previous techniques. Using overall habitat availability within a study area to estimate expected habitat use has been shown to be ineffective as the location where tracks are started, the duration of tracks, and the spatial distribution of habitat patches can have a large influence on the expected habitat use given no habitat preference by a tracked animal (e.g. Porter & Church 1987; Arthur *et al.* 1996). Arthur *et al.* (1996) and Hjermann (2000) attempted to alleviate this problem by defining a circle around each position (Arthur *et al.* 1996) or along a trajectory (Hjermann 2000), with a radius based on the maximum step that an individual is likely to make, and measuring relative habitat area inside this circle. However, doing so assumes that steps are equally likely in all directions and that maximum step distance is equal in all habitats. By using observed tracks and starting points, our randomization technique for assessing overall habitat preferences alleviates these problems as it makes no assumptions about the structure of the study area, or the directionality and distances of steps, and does not assume that starting points are random with respect to habitat.

The track randomization protocol is generally superior at detecting a known preference (or lack thereof) in simulated tracks than is comparison of observed use against overall habitat availability. The type I error rate (i.e. identifying a habitat preference when there is none) of the randomization procedure is comparatively low. In particular, when starting points are nonrandom, comparisons with overall habitat availability will often erroneously detect a significant preference for one habitat, while the track randomization procedure almost never will. This illustrates a critical problem with using overall habitat availability to test the null hypothesis of no habitat preference. In many studies, animals will be captured more frequently in one habitat, despite attempts to standardize sampling effort across habitats. For example, tiger sharks in Shark Bay are captured more frequently in deep habitats due to high rates of

bait loss in shallow waters (Heithaus 2001). In such situations, when starting position is not random with respect to habitat availability, the randomization procedure outlined here is particularly important.

The track randomization method can also be used to assess differences in habitat use between groups by testing the null hypothesis that observed differences in habitat use do not differ from that expected if neither group showed a preference (or showed identical preferences). This method provides a conservative test for differences in habitat preference. When identical preferences were programmed, the method correctly did not identify differences in preference. When differences in preference were distinct (i.e. one group prefers one habitat, the other group the other) this method also performed well. However, it was generally unable to identify correctly differences involving lack of preference by one group and a habitat preference by the other. It must be noted that the other two methods assessed also generally have difficulty detecting this difference. However, increasing the mean and maximum length of steps (which increases the probability of encountering both habitats) alleviates this problem somewhat and may account for its ability to detect a habitat preference in tiger sharks that rapidly move through their environment (Heithaus *et al.* 2002a).

The identity randomization technique is a more traditional randomization procedure. As such, it tests the same null hypothesis as parametric and nonparametric comparisons between groups, namely that there is no difference between habitats used in the two sets of tracks, not that there is no difference in preference. This method has the typical advantage of not assuming any particular underlying distribution, while having greater power than nonparametric tests. This method is generally better than track randomization at detecting differences between groups when one subgroup prefers a common habitat and the other has no preference, but is worse when one group has no preference and the other prefers a rare habitat. When the starting location of tracks is biased toward a habitat that neither group prefers, the identity randomization procedure is much better than the track randomization procedure at identifying differences.

The relatively good performance of identity randomization vs. track randomization at identifying differences between a subgroup with no preference and a subgroup with a preference for the generally more common habitat (first row of Table A2) probably reflects the conservative nature of the latter test (see Appendix I). If one-tailed tests are used, the proportion of correctly identified differences increases to 37·3% for track randomization and 44% for identity randomization. The nonparametric test remains poor with only 21% of cases correctly classified, even with a one-tailed test. The Mann–Whitney *U*-test is not necessarily conservative, however, because in comparisons where both groups have the same preference, it tends to incorrectly identify a preference more often than does track randomization.

These analyses suggest that randomization procedures, in general, may overcome some of the drawbacks associated with comparisons against overall habitat availability or parametric or nonparametric comparisons between sets of tracks. For the latter, identity randomization is the more powerful technique. However, if the assumption that starting locations are random with respect to habitat is violated, this method performs poorly. If starting locations are nonrandom, we recommend using the track randomization technique. Because this is a conservative test, we also suggest that separate track randomizations within each group (e.g. within males and within females) be performed if this test does not indicate a difference in preferences.

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Appendix I. Validation of randomization protocols

In order to test the power of our randomization techniques in detecting microhabitat preferences or differences in preferences among subgroups, we created sets of tracks with known habitat preferences and movement decision rules moving through simulated environments. The simulated environment measured 100×100 units and consisted of two habitat types ('1' and '2'). Any track leaving the environment re-entered at the corresponding location on the opposite side of the environment (i.e. a torus). For each simulation ($n = 2400$), three to eight blocks of habitat 2 were randomly generated. The dimensions of each block were obtained from a uniform distribution, with a range from 5 to 45 units. Therefore, habitat 2 was the less common habitat. We then generated either 40 (for measurement of overall habitat preference) or 80 (for comparisons between subgroups) simulated animals ('agents') moving through the environment (see Appendix II for details of movement rules). We simulated groups of agents that (1) had no underlying directional bias to their movements, or (2) had a strong tendency to move directionally regardless of habitat preference. For each combination of directionality and habitat preference (no preference, prefer habitat 1, prefer habitat 2), we created 75 unique environments each with a novel set of agents. Within environments created for tests of overall habitat preference we conducted 1000 randomization iterations and considered a habitat preference by the agents to be significant if fewer than 25 randomly generated habitat-use values were greater than the actual agent use of that habitat. We also performed binomial tests

Table A1. Test of *track reassignment* protocol to determine overall habitat preferences within simulated environments. Results of binomial tests on pooled data are shown for comparison. Numbers are the proportions of 75 replicate simulated environments in which habitat preference (or lack of preference) was correctly classified using two-tailed tests with $\alpha = 0.05$. Values that are significantly greater than that of the competing procedure are denoted with asterisks (Fisher's exact test; $P < 0.05$). The randomization procedure performed as well or better than the binomial test in most situations

	Randomization	Binomial test
Initial starting point random		
No directional bias		
no habitat preference	0·960*	0·427
preference for habitat 1	0·907	1·000*
preference for habitat 2	0·960	0·933
Directional bias		
no habitat preference	1·000*	0·520
preference for habitat 1	0·987	1·000
preference for habitat 2	0·987	0·973
Initial starting point in habitat 2		
No directional bias		
no habitat preference	0·960*	0
preference for habitat 1	0·947	0·880
preference for habitat 2	0·747	1·000*
Directional bias		
no habitat preference	0·987*	0·013
preference for habitat 1	0·987*	0·707
preference for habitat 2	0·987	0·987

on pooled data to assess whether observed habitat use differed from that expected using the overall frequency of each habitat in the simulated environment. For tests of differences in habitat preference among subgroups, we generated two subgroups ('A' and 'B'), each composed of 40 agents. We created 75 unique environments and groups of agents for each combination of preferences except for no preference/no preference using track reassignment and identity reassignment methods. Observed differences in use of a habitat between subgroup A and subgroup B within each simulated environment were compared with differences obtained from 1000 randomization iterations, and significance was determined as above. To compare these methods with a more traditional method, we used Mann–Whitney *U*-tests to determine whether observed habitat use by the two groups differed. The track reassignment protocol for assessing overall habitat preferences accurately detected habitat preferences or a lack thereof and performed as well or better than comparisons with habitat availability in all cases (Table A1), except when most tracks began in the preferred habitat (so that agents with preferences tended to move randomly within them). Track reassignment, identity reassignment or both generally out-performed the nonparametric test for differences in habitat preferences between groups, and all three tests were good at identifying differences, or lack of differences when both sets had a habitat

Table A2. Tests of *track reassignment* and *identity reassignment* protocols for detecting differences in habitat preferences between subgroups within simulated environments. All combinations were tested under conditions where the initial starting points of tracks were random with respect to habitat type or tracks all started in the rare habitat (habitat 2). Results are also shown for Mann–Whitney *U*-tests performed on the same simulated data. Numbers are the proportions of 75 replicate environments for each comparison in which the difference (or lack of a difference) in preference were correctly classified using two-tailed tests with $\alpha = 0.05$. Values for randomization tests that are significantly greater than those of other analysis techniques are denoted with asterisks (Fisher's exact test; $P < 0.05$). Mann–Whitney tests never outperformed randomizations

	Track reassignment	Identity reassignment	Mann–Whitney test
Initial starting point random			
No directional bias			
no preference vs. habitat 1	0·173	0·293	0·16
no preference vs. habitat 2	0·507	0·387	0·453
habitat 1 vs. habitat 2	0·907	0·913	0·867
habitat 1 vs. habitat 1	0·987	0·933	0·933
habitat 2 vs. habitat 2	0·907	0·96	0·96
Directional bias			
no preference vs. habitat 1	0·493	0·573	0·307
no preference vs. habitat 2	0·68	0·587	0·747
habitat 1 vs. habitat 2	0·98	0·993	0·967
habitat 1 vs. habitat 1	1	0·96	0·92
habitat 2 vs. habitat 2	0·933	0·867	0·933
Initial starting point in habitat 2			
No directional bias			
no preference vs. habitat 1	0·587	0·973*	0·973
no preference vs. habitat 2	0·32*	0·147	0·107
habitat 1 vs. habitat 2	0·98	1	1
habitat 1 vs. habitat 1	0·973	0·96	0·96
habitat 2 vs. habitat 2	0·987	0·92	0·88
Directional bias			
no preference vs. habitat 1	0·547	1*	0·973
no preference vs. habitat 2	0·453*	0·147	0·133
habitat 1 vs. habitat 2	0·98	1	1
habitat 1 vs. habitat 1	0·987	0·987	0·973
habitat 2 vs. habitat 2	0·973	0·933	0·92

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preference (Table A2). Compared with identity reassignment, track reassignment was better at identifying differences between a subgroup with no preference and a subgroup that preferred the generally rare habitat (habitat 2), but often worse at identifying differences between a subgroup with no preference and one that preferred the more common habitat (habitat 1). All three procedures had high Type II error rates, but such errors were reduced when the mean length of steps and maximum step length increased. For comparisons between no preference and a preference for habitat 1 with random starting positions and no directional bias (i.e. 1st row of Table A2), track randomization correctly classified 38·7% of preferences and identity randomization's performance improved to 46·7% when step lengths increased. The Mann–Whitney *U*-test remained comparatively poor even with longer step lengths, at 20% correct classification. A similar improvement with increasing step length was found for a comparison of no preference and preference for habitat 2, when tracks began in habitat 2 (12th row of Table A2; track randomization: 48%, identity randomization: 21·3%, Mann–Whitney *U*-test: 20%).

Appendix II. Movement rules for agents in simulated environments

Each agent made 15–20 steps based on a uniform distribution. For most simulations, agents moved 0–5 units ($\bar{x}$ = 2 units) in accordance with a Poisson distribution, or, if a habitat preference was defined for a set, the rules outlined below. To examine the influence of track characteristics on this technique, we also performed some simulations with a mean step length of 4 and a maximum step length of 8. In half of the simulations, we defined an overall directional preference where 75% of the 'east–west' component of a step were to the east and 75% of the 'north–south' component of a step were to the north. For the other half of the simulations, steps were equally likely in all directions. When a habitat preference was defined, an agent currently in the less favoured habitat would scan the maximum move distance in the direction dictated by the above rules. If it encountered the less favoured habitat at the expected destination, it did not move, but searched again (up to a total of three times), moving as soon as it encountered the favoured habitat or on the third unsuccessful search. Within the favoured habitat, an agent would move randomly until it left that habitat. A position was recorded at the end of each step.