Abstract

In this study, crayfish habitat distribution was studied in the laboratory setting on the northern crayfish (*Orconectes propinquus*), and the virile crayfish (*O. virilis*). *O. propinquus* is an invading species in northern Wisconsin and has successfully out competed and replaced *O. virilis* in areas they have been introduced. In this experiment, distribution of crayfish in four habitats: macrophytes in muck, macrophytes in sand, cobble and sand, were recorded twice during the day and night during the summer of 2004. There were four experimental treatments: *O. propinquus*, *O. propinquus* with predatory fish, *O. virilis*, and *O. virilis* with predatory fish. Predatory fish were smallmouth bass (*Micropterus dolomieui*), and rock bass (*Ambloplites rupestris*). It was hypothesized that in the presence of predators, during the day, the cobble habitat will be preferred by both species and at night the macrophytes in muck will be preferred. Also, during the day sediment will be preferred over macrophytes and at night macrophytes will be preferred. In the absence of a predator, crayfish will be evenly distributed among the two habitats containing macrophytes and cobble during the day and night and that more crayfish will be located on the macrophytes than on the sediment day and night for foraging. With the addition of a predator there was a shift of *O. propinquus* distribution to the cobble habitat during the day and to the macrophytes in muck habitat at night, but cobble was not the only significant habitat. The macrophyte in muck habitat was a significant habitat, but it was not the only significant habitat. There were no statistical differences found between distribution on the macrophytes and on the sediment in any case.

Introduction

The northern crayfish (*Orconectes propinquus*), has become successfully established in the Wisconsin area lakes (Garvey *et al*. 1994, Hill and Lodge 1994, Hill and Lodge 1999) and is an invasive species to the area. Since its introduction, *O. propinquus* has been able to replace the native virile crayfish, (*Orconectes virilis*) (Hill and Lodge 1999). Its natural habitat is found in the lakes and streams of the Great Lakes Basin and in the Ohio, Missouri and Mississippi river systems (Stein and Magnuson 1976).

Invasive species are a threat to biodiversity in an ecosystem in many cases. Though movement of any species is natural, this rate is heightened by human activity. Of the world's more than 500 species of crayfish, North America is home to 75% (Lodge *et al*. 2000). It is important to understand the biology and behavior of the invasive northern crayfish so that we may prevent its spread, manage its current population in invaded areas and encourage the native population of *O. virilis* to retain species diversity. This experiment investigates the habitat selection of *O. propinquus* and *O. virilis* and how predation affects this selection. This information will help further the understanding of the competitive relationship between these two species.

O. virilis and *O. propinquus* differ in body and chela size as well as aggressive and non-aggressive behavior. *O. virilis* are larger than *O. propinquus* at equal ages but *O. propinquus* males have larger and longer chela then *O. virilis* males or females of the same body size (Garvey and Stein 1993 and Garvey *et al*. 1994). Garvey (1994) found that predation pressure by largemouth bass *(Micropterus salmoides)* was greater on *O. virilis* than that of other two invasive crayfish in the northwoods when studied in the laboratory. It was suggested by Garvey (*et al*. 1994) that the behavior of the less aggressive *O. virilis* made it more susceptible to predation and a poor competitor for prime positions in the habitat. Hill and Lodge (1999) suggest that *O. virilis* populations are able to sustain themselves in environments with low populations of predatory fish if only in competition with *O. propinquus*. When habitats were limiting in the experiment, it was *O. virilis* which was excluded from the provided shelters by *O. propinquus* and *O. rusticus,* another invasive crayfish.

O. virilis were the only crayfish inhabitants in Trout Lake early in the 20th century. By 1971, *O. propinquus* had supplanted *O. virilis* in Trout Lake (Hill and Lodge 1999). Between 1971 and 1981, 99% of the crayfish population on the lake's eastern shore was composed of *O. propinquus*. However, *O. virilis* made a comeback by 1983 and composed 25-50% of the crayfish population. The relationship between *O. propinquus* and *O. virilis* is dynamic and requires more study to understand their interactions with their environment, with predators, and with each other.

Crayfish movement during the day and night are affected by predator presence. Stein and Magnuson (1976) found that in aquaria smallmouth bass, *(Micropterus dolomieui)* significantly affected the distribution, habitat preference, activity level and defensive behaviors (chela displays and burrowing) of *O. propinquus*. When predators were present, the crayfish moved to the area that offered them optimal shelter, preferring pebble to sand. These areas offer ample shelter, but food resources are few.

Another example of predation effects on crayfish behavior was an experiment on diel movement of crayfish species with and with out the presence of predators. Movement among habitats and habitat preference changed when congeners or predators were present (Hill and Lodge 1994). The preferred day time substrate was cobble. When congeners such as *O. rusticus* and *O. propinquus* were present *O. virilis* increased its use of the muck-macrophyte habitat and *O. propinquus* increased its use of the sand-macrophyte habitat in the presence of *O. rusticus* only. When only one species occupied a tank, crayfish were evenly distributed among all habitats except sand.

O. virilis and *O. propinquus* share the same habitat (Hill and Lodge 1994) and are thus in competition for resources and shelter. As their activities change between day and night, so do the resources they compete over. During the day, shelter is the most important resource to attain in order to escape predation and at night the two species may compete for food. As part of this study we examine the habitat preference of *O. propinquus* and *O. virilis* with and with out the presence of predators over day and night time hours in tanks with sand, cobble, sand with macrophytes and organic muck with macrophytes habitats as well as location within macrophyte habitats; resting on the macrophytes or on the sediment. It is hypothesized that, in the presence of a predator, during the day, the cobble habitat will be preferred by both species and at night the macrophytes in muck will be preferred due to the high organic detrital material found in muck with macrophytes. Also, that during the day the sediment will be preferred over macrophytes and at night macrophytes will be preferred for foraging. With a predator absent, it is hypothesized that crayfish will be evenly distributed among the two habitats containing macrophytes and cobble, as found in Hill and Lodge (1994), during the day and night and that significantly more crayfish will be located on macrophytes than on sediment, day and night, for foraging.

Methods

O. propinquus and *O. virilis* were held in circular flow-through tanks which each contained four distinct habitats; sand, cobble, macrophytes in sand and macrophytes in muck (organic sediments) to discern each species habitat preference with and without a predator present. Experiments were conducted at the University of Notre Dame Environmental Research Center, Land O'Lakes, Vilas County, Wisconsin, between June 15th and July 15, 2004. Each tank had a diameter of 1.82m and height of 0.66m and subject to a photocycle of 16 h light and 8 h dark (Garvey *et. al*, 1994). The water used in the tanks was a mixture of water from the epilimnion Tenderfoot Lake and ground water. Dissolved oxygen was kept above 6 mg/L and temperature was kept between 14-17°C. Dissolved oxygen and temperature were measured once a week.

O. propinquus (28-32mm carapace length [CL]) were collected from Hagerman Lake in Gogebic County, Michigan, and Tenderfoot Lake in Vilas County, Wisconsin and measured using vernal calipers. *O. virilis* (37-43mm CL) were collected from Forrest Lake and Crab Lake in Vilas County, WI. Crayfish were collected using minnow traps baited with 120 g of beef liver, set overnight. Only Form II male crayfish were used in all experiments. Crayfish were held in separate tanks prior to experiments and fed shrimp pellets and fish flake food once a day throughout experiment. They were each marked with orange spray paint for night time identification, a dot on the carapace of *O. propinquus* and a strip on the carapace of *O. virilis*.

The bottom of each tank was sectioned off in pie-slice like quarters and filled with sand, sand with cobble, sand with macrophytes or muck with macrophytes. Macrophytes were anchored using small pebbles. Sediments were approximately 4cm deep. All sediment, cobble and macrophytes were collected from Tenderfoot Lake. Thirty pieces of cobble, diameter 8-10 cm, were placed in one wedge of the tank with a total density of 22 pieces of cobble/m^2. Macrophyte species used were *Potamogeton amplifolius*, *P. gramineus*, *P. zosterformis*, *P. robbinsii*, and *P. richardsonii* at densities of 14.7, 29.4, 3.67, 0.7, and 13.95 shoots/m^2, respectively. Total plant density was 62.4 shoots/m^2.

After each tank was prepared, 20 *O. propinquus* were added to each of the sand quarters in three tanks and 20 *O. virilis* were added to the sand quarter in one tank. The crayfish were then observed and their location within the tanks and whether they were on the macrophytes or sediment was recorded at 0100, 0800, 1900 and 2300 hrs. Except during trials with a predator present, dead *O. propinquus* found were removed and replaced. Dead *O. virilis* were not replaced because of a short supply of that species in the area. During trials with a predator present, dead *O. propinquus* and *O. virilis* were removed but not replaced. Before any observations took place the crayfish were given 24 hours to disperse through out the tank.

Fish were caught using fyke nets set overnight in Tenderfoot Lake. Two species were used, one smallmouth bass (43 cm total length [TL]) and three rock bass, *Ambloplites rupestris*, (19.1 cm-20 cm TL). Each tank contained no more than one predatory fish at a time. Predators were given 24 hours for acclimation before observations resumed. The smallmouth bass was placed in a tank with 20 *O. propinquus*. Two of the rock bass were placed in two other tanks, each containing 20 *O. propinquus*. The tank with *O. virilis* contained 16 individuals after die off before the rock bass predator was added. Location of the crayfish and the fish were recorded four times a day for four days.

Smallmouth bass were hard to capture. Only one was caught and used in the experiment. The data found from tanks containing rock bass and smallmouth bass were combined although they were not in the same species or size range.

All statistics on were done on Systat. ANOVA and t-tests were used to find significance in habitat distribution. The number of crayfish in each habitat was converted to percent in each habitat.

Results

Habitat Preference of O. propinquus*: Predator Absent and Present*

Without the presence of a predator in tanks containing *O. propinquus*, there was no significant difference in the average percent in each habitat between day and night (p=0.236, multi-way ANOVA). When time of day, habitat preference, and predator presence were compared, there was no significant difference (p=0.122, multi-way ANOVA). When habitat types were compared there was no significant difference between the macrophytes in muck and macrophytes in sand habitats (p=0.184, ANOVA). There was a significant difference between macrophytes in muck and cobble (p<0.0001, ANOVA), macrophytes in muck and sand (p<0.0001, ANOVA), macrophytes in sand and cobble (p<0.0001, ANOVA), macrophytes in sand and sand (p<0.0001, ANOVA) and sand and cobble (p<0.0001, ANOVA) with day and night data combined.

In the presence of a predator, there was a significant difference in the average percent in each habitat between day and night (p=0.039, multi-way ANOVA) and between predator presence or absence and habitat preference (p<0.0001, multi-way ANOVA).

When day time habitat selections were compared there was no significant difference between the macrophytes in muck and macrophytes in sand habitats (p=0.625, ANOVA), macrophytes in muck and cobble (p=1.0, ANOVA), macrophytes in sand and cobble (p=0.599, ANOVA). There was a significant difference between macrophytes in muck and sand (p=0.001, ANOVA), macrophytes in sand and sand (p<0.0001, ANOVA) and sand and cobble (p=0.001, ANOVA).

When night time habitat selections were compared there was no significant difference between the macrophytes in sand and cobble habitats (p=0.83, ANOVA). There was a significant difference between macrophytes in muck and macrophytes in sand (p=0.016, ANOVA), macrophytes in muck and cobble (p=0.094, ANOVA), macrophytes in muck and sand (p<0.0001, ANOVA), macrophytes in sand and sand (p=0.001, ANOVA) and sand and cobble (p<0.0001, ANOVA).

Figure 1 refers to the difference in distribution of O. propinquus in the presence and absence of a predatory fish during the day. Distribution in the presence and absence of predatory fish at night is referred to in Figure 2.

Location within Macrophyte Habitats: O. propinquus

There was not a significant difference in the average percent of *O. propinquus* on plants and on the sediment (p= 0.602, t-test). Neither the presence nor absence of a predator (p=0.813, ANOVA) or the diel period (p=0.563, ANOVA) had a significant effect on distribution on plants or on sediment. When predator presence and time were combined the p-value was not significant (p= 0.813).

Fig. 1

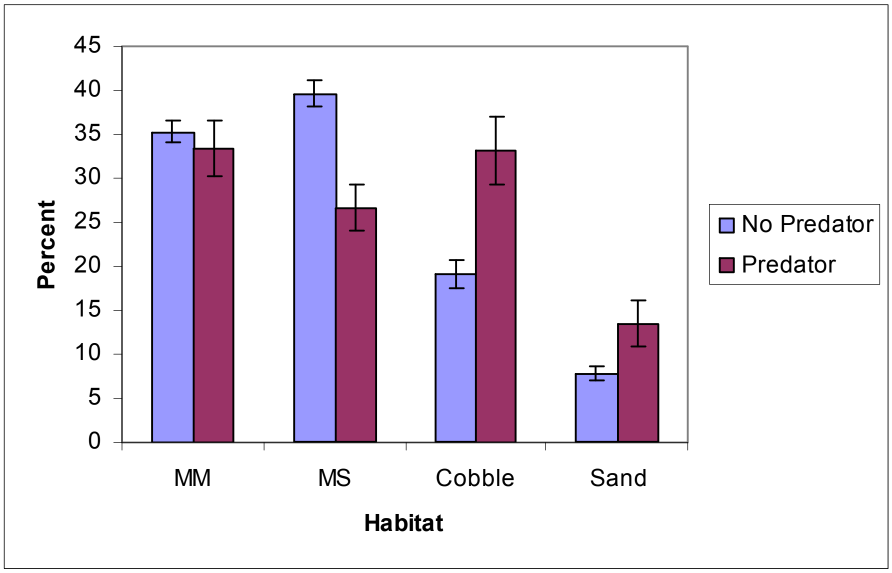

Figure 1. The average percent of *O. propinquus* in each habitat during the day with and with out a predatory fish. Abbreviations: MM-macrophytes in muck and MS- macrophytes in sand

Fig. 2.

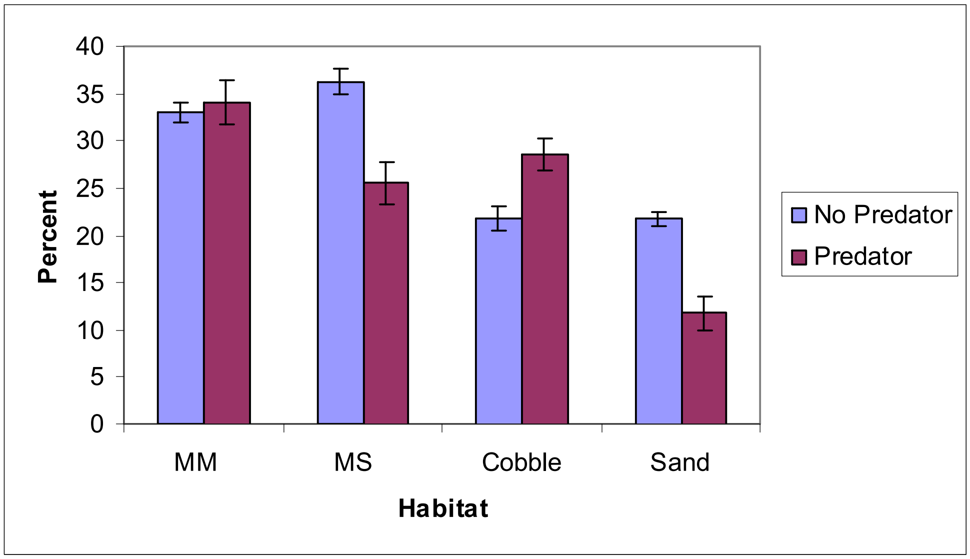

Figure 2. The average percent of *O. propinquus* in each habitat at night with and with out a predatory fish.

Habitat Preference O. virilis*: Predator Absent and Present*

In the absence of predators and during the day the average percent of *O. virilis* in macrophytes in muck was the highest (44.91%), followed by macrophytes in sand (34.06%), cobble (18.26%) and sand habitats (11.68%). This also held true at night, though the average percent of *O. virilis* in cobble went up by 2.92% while the average percent in the other three habitats declined by approximately 4%.

During the day and when predators were present, the average number of *O. virilis* in the cobble habitat is 84.3%, followed by macrophytes in sand (28.17%), macrophytes in muck (25.60%) and sand (0.96%). At night the cobble habitat contained 38.14% of the total population followed by macrophytes in sand (28.81%), macrophytes in muck (14.62%) and sand (10.08%).

Figure 3 refers to the difference in distribution of O. virilis in the presence and absence of a predatory fish during the day. Distribution in the presence and absence of predatory fish at night is referred to in Figure 4.

Location within Macrophyte Habitats: O. virilis

During the day and in the absence of a predator, there was a higher average percent of *O. virilis* found on the sediment (65.08%) than on macrophytes (34.92%). At night there was a higher average percent on macrophytes (54.83%) than on the sediment (45.17%).

Fig. 3.

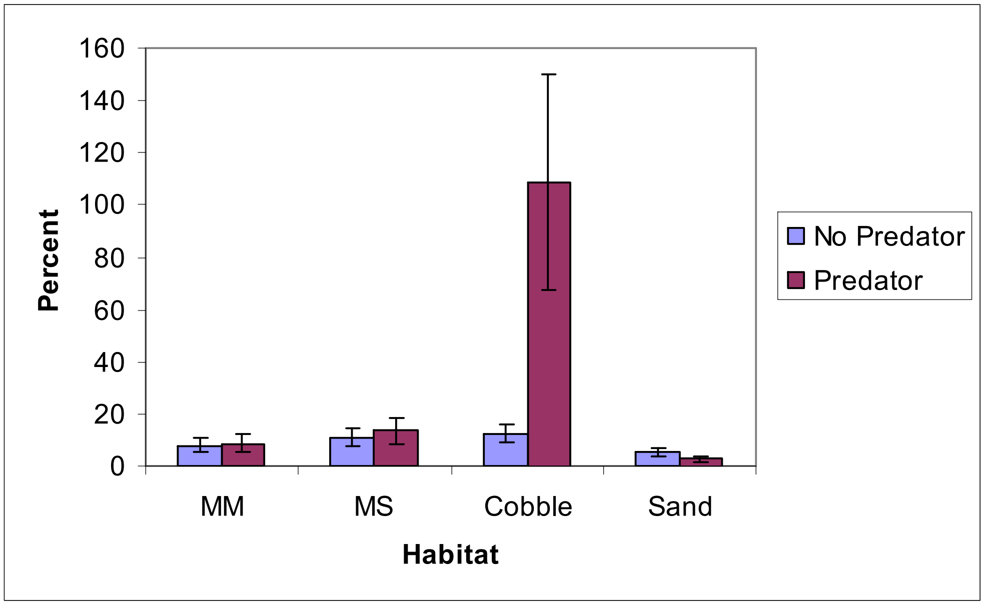

Figure 3. Distribution of O. virilis among four habitat types during the day comparing predator present and absence.

Fig. 4.

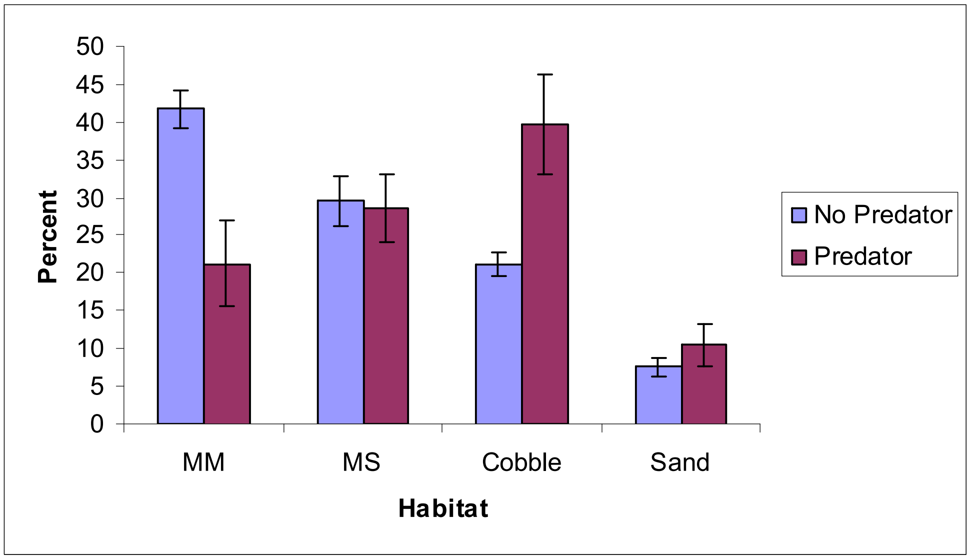

Figure 4. Distribution of O. virilis among four habitat types at night comparing predator present and absence.

During the day and in the presence of a predator, there was a higher average percent of *O. virilis* found on the sediment (75.45%) than on plants (24.55%). At night the average percent on sediment was 51.44% and 48.56% on the plants.

Discussion

O. propinquus has been found to successfully replace *O. virilis* in most situations (Hill and Lodge 1999). It has been proven that when *O. propinquus* is introduced, *O. virilis* shifts its habitat use (Hill and Lodge 1994). This experiment explored the habitat preference of *O. propinquus* and *O. virilis* and how this changes when in the presence of a predatory fish.

While in a tank with no predator present, *O. propinquus* was not evenly distributed throughout the four habitats available during the day (Fig. 1). They were distributed mainly through out the half of the tank with macrophytes and the cobble section, presumably because there is more food available in the sections containing macrophytes and shelter in the cobble habitat. With no predator present they are able to move freely through out the tank. Fish flake food and shrimp pellets were added randomly to each section daily. This would also present the cobble habitat as an opportunity to find food and shelter.

While in the presence of a predator during the day (Fig. 1), *O. propinquus* exhibited a significant difference in habitat distribution. Contrary to Hill and Lodge's (1994), findings that *O. propinquus* prefers cobble when in a tank with a predator and no other crayfish species, this study found that *O. propinquus* were contained in the macrophyte and cobble sections followed by sand.

At night, there was no significant difference between macrophytes in sand and cobble and between macrophytes in muck and cobble, although this was very close to being significant (p=0.094). In this situation the macrophytes in muck and cobble habitats were preferred (Fig. 2). Both of these areas provide shelter, but in differing ways. The muck is softer and less compact than sand and may be easier to burrow in, giving the crayfish protection from predation.

O. propinquus distribution within the two macrophyte habitats was found on the macrophytes themselves and on the sediment. This was thought to be an effect of predator avoidance and foraging. If this were the case, there would be more crayfish on the sediment, where there may be some shelter from predators, than on the macrophytes during the day in the presence of a predator. This is not what was found. There was not a significant difference between the average percent of crayfish within that habitat on macrophytes and on the sediment during the day, night, with a predator or with out a predator in the tank. The distribution on the macrophytes and sediment was not affected by any of the trial factors.

These results may be affected by changes in behavior of the fish predator and *O. propinquus* when place in an aquarium setting. There was never an observation of a predator catching or attempting to catch a crayfish during the experiment. It may be that the predatory fish did not pose enough of a threat to elicit a significant change in crayfish behavior from trials with out a predator to trials with a predator present. It may also be the case that attaching to the macrophytes provides the same amount of shelter as remaining on the sediment.

There was one trial run with *O. virilis* in this experiment and the dead were not replaced. Not enough *O. virilis* were able to be caught due to small local population sizes to run a trial on any more than one tank. No true conclusions can be drawn from the *O. virilis* data. A study on the local populations of *O. virilis* and *O. propinquus* would be interesting for further study.

No statistical tests were able to be run on this data; however, there were trends in *O. virilis* behavior which may be validated with more replicates. When a predator was absent *O. virilis* distribution appeared to be similar to that of *O. propinquus* where the majority of crayfish were found in the two quarters containing macrophytes, followed by cobble then sand habitats. The two macrophytes sections contained the highest amount of food available and with no predation threat would attract crayfish.

While a predator was present the majority of *O. virilis* were in the cobble habitat during the day (Fig. 3). Since more than 80% of the population in the tank was found in the cobble habitat during the day, it would seem that the predator present had a large effect on behavior. The percent found in the cobble section went down by 46.16% at night when predation threat is minimal (Fig. 4).

In this experiment, *O. propinquus* favored three habitats; macrophytes in muck, macrophytes in sand and cobble. The most interesting pattern in their behavior is the difference in distribution in during the day in tanks with and with out predatory fish. It is during the day that crayfish are most vulnerable to predation by visual predatory fish like smallmouth bass and rock bass. When there is no predation threat, *O. propinquus* gather in the two macrophyte sections. After a predator was added to the tank, the distribution shifted slightly to include cobble as a significantly used habitat.

The hypothesis on the addition of a predator shifting *O. propinquus* distribution to the cobble habitat during the day and to the macrophytes in muck habitat at night was not completely proven. While cobble did become a significantly used habitat, it was not the only significantly used habitat. The same occurred for the macrophytes in muck habitat. The habitat containing macrophytes in muck was a significantly used habitat, but it was not the only significantly used habitat.

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