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Preference for Artificial Refugia over Natural Refugia in an Endangered Fish

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Abstract: The availability of appropriate refugia may ameliorate some impacts of habitat degradation and can aid in the conservation of target species. In addition to natural refugia, the provision of artificial refugia may be viable, particularly in degraded habitats. We examined the conservation potential of natural and artificial refugia for the endangered Eastern Cape redfin, *Pseudobarbus afer*. We show that deeper water is preferred so is likely to constitute a natural refuge. However, this preference is overridden by the provision of artificial refugia (a plastic pipe). We highlight that the most important habitat factor in the conservation of *P. afer* is availability of suitable natural refugia through avoidance of habitat destruction. However, when redfin habitat is already compromised, appropriate artificial refugia may supplement the protection provided by natural refugia and may provide benefits to the whole aquatic community.

Keywords: Eastern Cape redfin; *Pseudobarbus afer*; habitat degradation; deep water refuge; South Africa

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1. Introduction

Freshwater ecosystems are vitally important yet are among the most degraded in the world [1–3]. Two of the five greatest threats are flow modification and habitat destruction [3–5], with consequent impacts on biodiversity [1–3,6,7]. One factor that may ameliorate some impacts of habitat degradation is the availability of refugia e.g., [6,8–10]. Unfortunately, habitat alteration itself can reduce the extent of refugia [6], which, in turn, increases the vulnerability of shelter-using species to predation and stochastic events [6,11,12]. Despite this, awareness of the roles of refugia in aquatic systems is inadequate [6,9,11,13].

Refugia may be broadly defined as all habitats and environmental factors that temporally or spatially reduce the negative effects of biophysical disturbances compared to surrounding places or times [11]. Although refuge preference and use tend to be species-specific, any type of habitat heterogeneity generally provides a refuge for some species [8,10,14,15], and selection of appropriate refugia can aid in the conservation of target species [9,13]. Natural freshwater refugia may be generated by differences in flow regime e.g., [16,17] or consist of biotic features such as vegetation e.g., [18]. An alternative option that may be viable, particularly in already degraded habitats, is the provision of artificial shelter [6,8,10,13]. Artificial refugia may take a variety of forms, including plastic pipes e.g., [6,19], artificial vegetation e.g., [8], and introduced boulders or deadwood e.g., [10,15].

In this study, we examined the use of natural and artificial refugia by a small (approximate total length: 5–6 cm, body height: 1 cm; Figure 1) endemic cyprinid minnow, the Eastern Cape redfin, *Pseudobarbus afer* (Peters 1864). Redfins inhabit the many streams in the Eastern Cape, where they feed on filamentous algae and invertebrates [20]. As direct testing of refugia effectiveness using predators is precluded by redfin's endangered

status [21], we assumed a refuge used in the absence of a direct threat would likely be utilized in a threatening situation as well. A major requirement of redfin habitat is sufficient shelter options [22,23]. Water abstraction [4] and drought [11] are common causes of habitat degradation that reduce the availability of refugia. South African rivers, including those inhabited by redfins, are heavily affected e.g., [24–26]. As one effect of both water abstraction and drought is reduction in water level, we first asked whether this would constitute a reduction in the availability of natural refugia by assessing redfin's preference for deeper water. Then, as redfins' preferred response to predator stimuli is to hide, even in artificial refugia [27], we assessed whether redfins would use artificial refugia in normal circumstances (i.e., without a predation threat), and to what extent artificial refugia would be used and would, therefore, be potentially useful in protecting this species. We hypothesized that redfins would use artificial refugia, but that they would prefer deeper water, and this preference would be maintained even when artificial refugia were provided.

Figure 1. Three examples of *Pseudobarbus afer* specimens. The scale bar = 10 mm.

2. Materials and Methods

Redfins were collected using a dip net from pools in a 2 km stretch of the Klein Uie River (Le Roux farm; 33°19.825′ S, 25°29.456′ E). The fish were maintained for 1 month to acclimate to experimental conditions, during which they were fed once or twice per day on tropical flake fish food.

Seven 1 m diameter pools were constructed from 500 µm black PVC plastic supported by 30 cm high corrugated plastic sides. Each pool contained a 3 cm thick layer of cleaned sand and 80 l of river water. A domed, semi-circular enclosure, approximately 2 m tall at the highest point, was constructed, using off-white PVC plastic piping and green shade cloth, and placed over the pools. The enclosure was open at each end, allowing air to flow through, while the shade cloth roof mimicked overhanging riparian vegetation (Figure 2). This enabled us to maintain natural environmental conditions as much as possible.

Figure 2. Photograph showing the natural laboratory.

Two of the pools were stock pools to house the fish before and after the experiment. In the remaining five pools, aquaria (91 × 33 × 35 cm) were placed at different orientations. Using double pools allowed water temperature to be maintained at a more constant level. Each aquarium was divided into three sections using glass dividers. The outer sections measured 35 × 33 cm, while the inner section was 21 × 33 cm. Each glass divider had a 5 × 3 cm hole cut in it, 3 cm from the bottom and 14 cm from either side. The aquaria were filled with water to 12 cm in height. Sand substrate was placed in each outer section. In one section, the sand was level with the bottom of the hole in the divider on one side and sloped down to 0.5 cm deep adjacent to the sides of the tank. In the other section, the sand was again level with the divider hole on one side and sloped up to 8 cm deep at the outer edges. In both sections, the slope was the steepest that could be maintained so that most of each section contained substrate at a uniform height. This resulted in one side with water 9–11.5 cm deep (deep) and one side with water 4–9 cm deep (shallow). This was the maximum difference in water depth that could be maintained while ensuring the entrance from the centre to each outer section was as similar as possible (Figure 3).

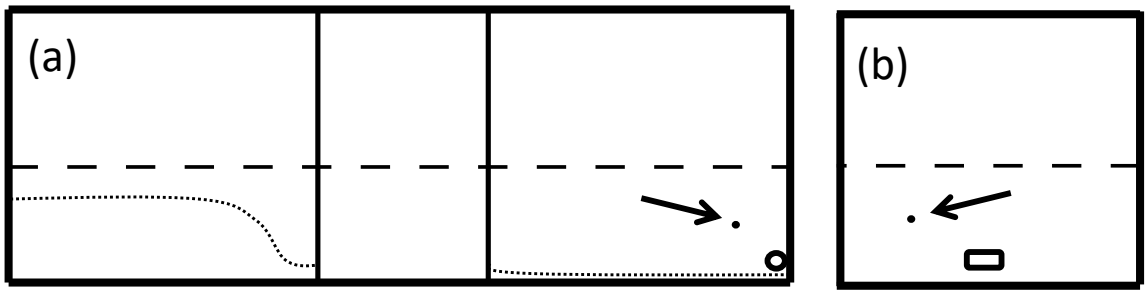

Figure 3. Schematic diagram of the aquaria set up, approximately to scale. (**a**) View of the long side of the aquarium; (**b**) view of the short side of the aquarium showing the position of the hole within the glass dividers. The dashed line represents the water level, the dotted line represents the height of sand within the two outer compartments, the circle shows the position of the refugia when present. The small black dots indicated by arrows provide an idea of the height of the fish relative to the aquaria.

The evening before a set of trials, one redfin was placed in the central area of each aquarium and left overnight to settle. The following morning, a refugium, consisting of a 10 cm long white PVC pipe with an internal diameter of 3 cm, was placed against the short side of the shallow section of some of the aquaria. Previous work showed redfins will readily use such artificial refugia [27]. The fish were fed and then monitored at regular intervals (at least 6 times), noting their position within the aquaria. After 1 h, refugia were carefully upended to encourage fish to exit if necessary, and fish were gently herded to the central area using a dip net. As soon as the fish reached the central area, the dip net was removed, and trials began. For each fish, position within the tank was recorded at 0, 30, 60, 90, and 150 min after the net was removed. Every care was taken to ensure fish were not disturbed during observations. At the end of this time, refugia were removed and the fish were again left in the aquaria overnight. The following morning, the same procedure was repeated, placing refugia in the aquaria that were without refugia the previous day. Each fish was thus subject to two trials, one with and one without refugia, after which they were placed in a separate stock tank so that they were not resampled. The shallow and deep sides of each aquarium were swapped between fish and the order of refuge/no refuge was randomized for each fish. In total, 25 fish were observed.

Two of the fish were always observed in the same position within the central area of the aquarium in both trials, and a further 5 fish failed to move in one of the trials, even when presented with food. The fish were thus considered unable to behave normally and these trials were excluded from the analysis. Analysis was performed via a generalized estimating equation (a special case of general linear model for use when data are not independent; see e.g., [28]). The within-subject independent variables were treatment (with/without refugia) and position (central, shallow, deep). Individual fish number was included in the model as a between-subjects factor. The dependent variable was the frequency with which fish were found in each area (Poisson distribution, log-link function). The analysis was conducted using IBM SPSS version 20.

This study followed all national guidelines for ethical research and was approved by the Rhodes University Ethics Committee (no reference number). The Department of Economic Development and Environmental Affairs issued the permits necessary for fish collection (CRO 192/08CR and CRO 193/08CR), and Abraham and Pieter Le Roux allowed access through their property to collect fish from the Klein Uie River. The data from this study are available upon request to the corresponding author.

3. Results

Most of the fish readily used the refugia provided. Of the fish included in the analysis, 29.3% were observed in all three sections of the aquaria, 36.6% were observed in two sections, and 34.1% in one section. However, when the initial 1 h settling time was included, these values changed to 42%, 40%, and 18% respectively, and all fish were assumed to have visited all sections of the aquaria between being placed in the aquaria and being fed the following morning. In many cases, the fish immediately swam through one of the entrances when the net was removed, while in other cases, the fish took more time to move. The frequency of times observed in each area differed significantly with/without refugia (Wald $\chi^2 = 6.59$; df = 1; $p = 0.01$), and in both treatments, increased significantly from shallow though central to deep sections of the aquaria (Wald $\chi^2 = 15.70$; df = 2; $p < 0.001$). However, the significant interaction effect (Wald $\chi^2 = 7.77$; df = 2; $p = 0.021$) highlights an interesting relationship. When there was no refuge available, fish spent more time in deeper water, but when refugia were present, the time spent in deeper water decreased in favour of the shallow section where the refuge was placed (Figure 4).

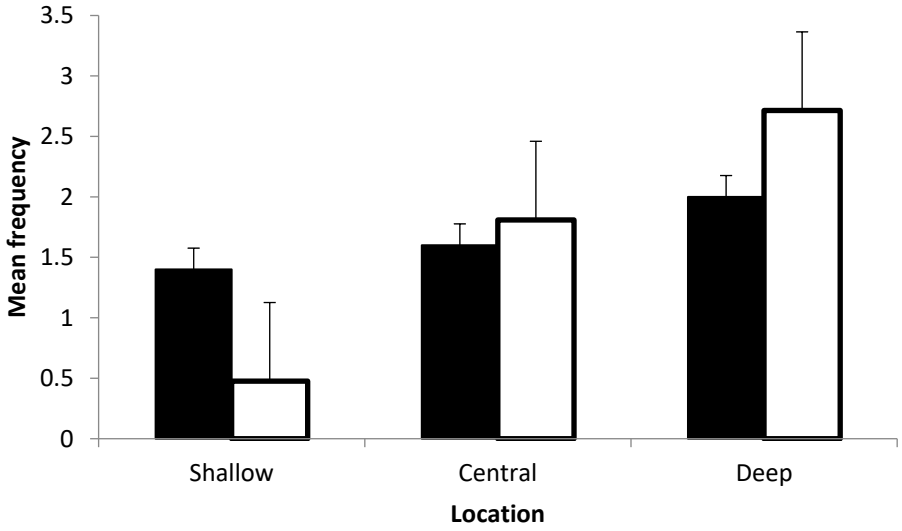

Figure 4. The mean frequency at which fish were found in each section of the aquaria at the 5 recorded observation times (minimum = 0, maximum = 5): black bars—with refugia; white bars—without refugia. Error bars represent standard error.

4. Discussion

Redfins used both the natural (deeper water) and artificial (plastic pipes) refugia. Field studies have shown that redfin populations are usually associated with refugia [22,23], which suggests that refuge use is characteristic of this species. Although a preference for deeper water does not necessarily mean that deeper water constitutes a refuge, it is likely that when under threat, redfins would flee to their preferred habitat for refuge, which is consistent with field observations (KM pers. obs.). Moreover, deeper water would provide a refuge from avian predation [23]. It must also be noted that although the differences in depth in this study amount to just a few centimetres, in terms of the perception of a small fish, this is a large difference; in human terms, it is equivalent to the difference between a two-story house and a six-story apartment block. Research into depth as a refuge in freshwater ecosystems is limited and mostly of concern in flood- or drought-prone areas. For example, although many interacting factors affect variation in fish stocks in the Mekong River in Laos, depth is considered the most important as a dry season refuge for fish [29]. There is more information from the marine realm. One study in Brazil showed that fish on shallow reefs were generally more wary of approaching humans than those on deeper reefs, suggesting fish treated deeper reefs as a safer refuge [30]. However, responses were both species- and size class-specific, indicating that deeper water is not necessarily best in all circumstances [30]. In addition, natural conditions are much more complicated than can be captured in controlled laboratory environments, so caution should be used in extrapolating our results to natural ecosystems. In any case, the clear preference for deeper water, even with the minimal differences in depth used here, indicates that in natural conditions, water abstraction and consequent lower water levels are likely to be highly detrimental to redfins.

While it is preferable to maintain natural refugia, such as deeper water, loss of such natural refugia can be at least partially ameliorated by the provision of artificial refugia. Redfins readily used the plastic pipes provided and their preference for the artificial refugia even overrode their preference for deeper water. Other fish species have also been found to use artificial refugia, though not all species are likely to benefit. Of nine species observed in a study from Brazil, only three cichlid species were consistently associated with a refuge made of plastic pipes and artificial plants [8]. Interestingly, in this study, substrate-located structures were overall preferred, but depth preferences were also species-specific [8]. The use of artificial refugia is also likely to be influenced by the function for which they are needed. While avoidance of predation is assumed to be a primary function of refugia [9,27], they can serve other purposes too. For example, in a native–invasive pair

of fish species (*Aphanius iberus* and *Gambusia holbrooki*, respectively), the native species used artificial refugia more, which helped alleviate aggressive harassment from the invasive species [13], but did not prevent sexual harassment of females of either species by males [31]. However, even in this case, there may be other benefits of refugia use, for example, to reduce the metabolic costs associated with predation avoidance mechanisms, such as camouflage [6,32].

The provision of refugia is one of a suite of potential mitigation approaches to deal with the multiple interacting stressors that redfins face. In addition to habitat degradation, an anthropogenic stressor of particular concern is the threat from invasion by novel predators [20,22]. This represents a "double whammy" for redfins—habitat alteration is likely to reduce refuge availability, and at the same time, may favour the survival of invasive species over native species [6,13,31]. Increasing the availability of natural or artificial refugia is, thus, likely to benefit redfins in invaded habitats. Another anthropogenic change of global concern is the climate crisis, which is also likely to interact with both habitat degradation [6] and invasive species [33] and complicate their impacts e.g., [1,3,34]. South Africa, including redfin habitat, is no exception to the impacts of climate change, and tropical to sub-tropical, small-bodied fish with limited distributions, such as redfins, are particularly vulnerable to climate change effects [35]. Deeper water may act as a thermal refuge [36] and artificial refugia can mitigate some impacts of habitat destruction [6] and provide protection from invasive predators [6,13]. Integrating these mitigation factors with others across freshwater, marine, and terrestrial systems will be more effective than using them in isolation [34]. Other species in the community may also form part of this "mitigation suite". For example, redfins interact with other small minnows within their habitat and react to alarm chemicals produced by con- and hetero-specifics by fleeing to refugia [27]. The provision of refugia may thus benefit other species as well, which can provide protection for redfins in turn. Assessing the interactions among refuge use strategies, various anthropogenic stressors, and the potential interacting solutions would be informative.

The Eastern Cape redfin is endemic to the Eastern Cape of South Africa and listed on the IUCN Red List as endangered, so would benefit from the conservation of existing populations [22,23] and assisted reintroduction. However, recent evidence separates the redfin populations used in this study into a distinct lineage (the Mandela lineage; [37]), restricting the species' distribution even further. Research into the potential conservation of this species is, thus, vital, and refugia are an important facet of conservation. The preservation of natural refugia is preferable, but in situations in which this is not possible or faces limited success, supplementing natural refugia with artificial, such as the plastic pipes used in this study, is a potential (and cheap) option [13,27]. This is one of very few studies examining the role of water depth and the use of simple artificial structures as refugia. More research is needed on refugia in general [6,9,11,13] and in aquatic ecosystems in particular [9], and we have highlighted several potential avenues of future research. In conclusion, both natural and artificial refugia can aid in the conservation of the Eastern Cape redfin, and likely the conservation of associated species and ecosystems as well.

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Data Availability Statement: Data used in the study is available upon request to the corresponding author.

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