Shade as enrichment: testing preferences for shelter in two model fish species

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Abstract

We compared preferences shown by zebrafish *Danio rerio* and three-spined stickleback *Gasterosteus aculeatus* for shelter provided by above-tank shade and artificial plants. Zebrafish showed no preference for either shelter, whereas sticklebacks showed a preference

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for both shelter types over open areas and for shade over plants. Our results suggest shade may be used as enrichment for captive fish and re-emphasise the importance of species-specific welfare considerations.

KEYWORDS

environmental enrichment, fish husbandry, fish welfare, three-spined stickleback, shelter, zebrafish

Ethical statement

All procedures performed were in accordance with the ethical standards of the University of St Andrews and methods used were approved by the University of St Andrews Animal Welfare and Ethics Committee (AWEC). No procedures required UK Home Office licensing. No fish died or suffered ill health during this study and all individuals were retained in the laboratory for future use.

It is widely understood that enrichment of the captive environment is beneficial both in terms of welfare of captive animals (Mellen & MacPhee, 2001) and ensuring reliable and biologically valid research outcomes (Newberry, 1995). Several calls have been made for evidence-based identification of biologically meaningful aspects and standardisations of enrichment used across studies and species of fish (Huntingford *et al*., 2006; Brydges & Braithwaite, 2009; Näslund & Johnsson, 2014). This is important not only for the broad variety of fish used as model species in biomedical, physiological and behavioural research

(Huntingford *et al*., 2006; Ashley, 2007; Williams *et al*., 2009; Sneddon *et al*., 2017; Browman *et al*., 2018) and may also be useful for ornamental fish in captivity worldwide ((Ploeg *et al*., 2007); see also Stevens *et al*., (2017)).

Environmental enrichment, both as a term and in practice, can take many forms and is often applied vaguely or inconsistently across studies - even within a well-studied species such as the zebrafish (Lawrence, 2007; Lidster *et al*., 2017). It should also be noted that enrichment preferences may vary between species (Kistler *et al*., 2011). Frequently, environmental enrichment is divided between social enrichment (typically provision of companions) and structural–physical enrichment (typically an increase in environmental complexity). The latter can improve physiological and psychological well-being of captive fish by reducing stress (Näslund *et al*., 2013), reducing aggression (Torrezani *et al*., 2013) and even improving cognitive performance and neural development (Kotrschal & Taborsky, 2010; Strand *et al*., 2010; Spence *et al*., 2011; Ebbesson & Braithwaite, 2012).

Despite the known benefits of physical enrichment, it may be costly or inconvenient to provide (Lidster *et al*., 2017). This has led to recent studies exploring possibilities for easily-implemented and low-cost alternatives, such as the exploration of the use of frequent water changes (Lee *et al*., 2018). Any standardised type of enrichment should strike a balance between maximizing the welfare benefits to the fish, while maintaining practicality and cost-effectiveness within a research environment.

We focus here on one aspect of physical enrichment: shelter. Areas with physical enrichment (particularly structural complexity) can provide refuges or shelter for fish in aquaria. Provision of this shelter, in the form of artificial or natural plants or other simple shelters, can be a low-cost and easily-maintained method of improving fish welfare in captive environments. Such shelter can provide refuge for fish from aquarium lighting (Boeuf & Le Bail, 1999; Marchesan *et al*., 2005), refuge from intra or inter-specific aggression (Näslund & Johnsson, 2014) and water currents (Webb, 2006), or as places to recover from sources of handling or other stress (Marcon *et al*., 2018). While many fish species spend more time in enriched areas when provided (Kistler *et al*., 2011; Schroeder *et al*., 2014; Sullivan *et al*., 2016), more work will be useful in understanding their preferences between and usage of, different types of shelter. For example a recent study showed goldfish *Carassius auratus* (L. 1758) had no preference between artificial and live vegetation (Sullivan *et al*., 2016).

In this study we investigated the preferences between two types of shelter, artificial plants and shade (provided by an external partial tank cover), in two commonly-used model fish species: the zebrafish *Danio rerio* (Hamilton 1822) and the three-spined stickleback *Gasterosteus aculeatus* L. 175. We used a behavioural assay with three conditions: (1) open, (2) plant and (3) shade, across three treatments to establish which enrichment these species prefer over a fixed period following transfer into a new tank and hence which are likely to be of the most value in improving the welfare of these species in captivity.

We used adult AB strain wildtype short-fin *D. rerio* from the University of St Andrews population in this study. Fish were housed in mixed-sex groups of 36 in 54 l tanks (60 x 30 x 30 cm) at mean ± SD 26.5 ± 0.4°C on a 12 L:12 D photoperiod cycle. Fish were fed daily to satiation on a commercial flake (Tetramin tropical flake, Tetra; www.tetra.net) and freeze-dried bloodworms. The aquaria contained 1cm gravel substrate, plastic and live plants (java moss *Hypnaceae* sp) and water quality was maintained using internal biomechanical filters as well as weekly monitoring (nitrate, ammonia, pH) and water changes.

We also used wild-caught *G. aculeatus*, collected from the Kinnessburn stream (St Andrews, UK) using scoop nets in July 2018 and housed in aquaria for 1 month prior to experimentation. Fish were housed in mixed-sex groups of 30 in 95 l tanks (90 x 35 x 30 cm) at mean ± SD 10.0 ± 0.6°C on a 12 L:12 D photoperiod cycle. Fish were fed daily to satiation with bloodworms. The housing aquaria contained a layer of gravel, artificial plants and were equipped with external filters to maintain water quality. Only adult, non-reproductive individuals were used in the experiment; individuals were not sexed.

The experimental setup was also the same for both species: a rectangular tank (100 x 25 x 25 cm) filled with the appropriate temperature of water was then set up for testing as per relevant experimental treatment (Figure1a). The tank was arranged such that two end zones, each accounting for 40% of the area of the tank, were presented as either shaded, plant, or open, in one of three treatments: shade–open, plant–open, or choice (shade–plant). Shade was provided by an opaque black plastic rectangle (cut to cover one of the end zones) and placed on top of the tank. The plants used were always the same set of three plastic plants, arranged such that they covered *c.* 50% of the surface area of the end zone they were placed in. The tank was lit from above with standard aquaria lights used in the fish laboratory; the tank was covered on three sides with opaque black plastic to reduce external disturbance.

Both species were subjected to the same procedure where fish were fed in their housing tanks 2 h prior to experiments and were tested in groups of 3 using individuals from the same housing tank; each fish was tested only once before being transferred to a different housing tank to avoid repeat testing. Each group of three fish (ten groups per treatment, for a total of 90 fish per species) were introduced into the middle zone of the experimental tank at 09:30 hours and left until 12:00 hours (midday), during which time a camera (ELP 2 Megapixel USB webcam; www.webcamerausb.com) recorded all movements. The trial was then terminated and the fish were returned to the housing aquaria. The experimental tank was then drained and refilled with conditioned water and re-arranged at least 1 day before the next group of fish was tested. The designation of each zone as either plant, open or shade was pseudo-randomised such that each group of fish was randomly assigned to a treatment, but across all treatments each shelter type was presented on the two end zones an equal number of times.

Scan counts of the number of fish in each zone were obtained from the video recordings at 5 min intervals throughout the experiment. These counts were then used to calculate preference index for each zone–treatment over the duration of the experiment using the Jacobs' preference index (J), as used in similar preference studies (Kistler *et al*., 2011; Schroeder *et al*., 2014; Jones *et al*., 2018; DePasquale *et al*., 2019). The Jacob's index provides values ranging between +1 (maximum preference) and –1 (maximum avoidance), with 0 indicating no preference (Jacobs, 1974) as: $J = (r - p)[(r + p) - 2rp]$ where $r = n_{area} n_{total}^{-1}$, with n_{area}= number of fish in the focal area, n_{total} = number of fish in the tank and p is the available proportion of the focal area out of the total space available in the aquarium (here $p = 0.40$).

All data were analysed in R (www.r-project.org). As the data were not normally distributed (Shapiro-Wilk test $W = 0.77514$, $P < 0.001$), we used a non-parametric Wilcoxon rank-sum test to compare Jacobs scores for the plant *v*. shade zones in the choice experiment; to explore effect of treatment on shelter type we used a nonparametric mixed-effect model with group as a random effect for each species and the lsmeans package in R (Lenth *et al*., 2018) for *post hoc* analysis.

Danio rerio showed no preference for either of the shelter types over the open area, instead displaying avoidance of both sheltered areas with lsmeans estimates of shade: $J = –0.33$ and plant: $J = –0.10$ (Figure 1b). In the choice treatment *D. rerio* showed no significant difference in preference between the shelter types ($W = 2512$, $P > 0.05$; Figure1b). *Gasterosteus aculeatus* conversely showed a preference for both types of sheltered areas over the open areas (shade: $J = 0.34$; plants: $J = 0.19$). In the choice treatment *G. aculeatus* showed significantly greater preference for shade over plants (Wilcoxon rank sum test, $W = 779.5$, $P < 0.05$; Figure1c).

In this study we looked for evidence of preferences for two types of physical enrichment, shade and plants, in two model fish species, *D. rerio* and *G. aculeatus*. We found that while *D. rerio* show avoidance for sheltered areas of a tank over open areas, *G. aculeatus* preferentially spend time in sheltered areas. When given both types of enrichment, *G. aculeatus* appeared to prefer shade over plants. The preference of wild-caught *G. aculeatus* for shelter is unsurprising, but their bias towards shade rather than plants may seem counterintuitive. This could be linked to the function of the shelter for a species such as *G. aculeatus*, which has a wide variety of predators, both aerial and aquatic. Above-water shelters may provide protection from avian predators such as heron or kingfishers (Wootton, 1984), while submerged plants may only serve as cover for aquatic ambush predators, such as pike *Esox Lucius* L. 1758 (Savino & Stein, 1989). Regardless of the reason, providing a shaded area may be a useful and easily-achieved improvement to the physical enrichment of their tanks.

Danio rerio, on the other hand, showed no preference for either shelter type, instead displaying avoidance of both shade and to a lesser extent, plants. This is consistent with previous work demonstrating that *D. rerio* showed reduced preferences for structured areas in an aquarium compared to other species (Kistler *et al*., 2011) and that wild *D. rerio* occur as frequently in open water as they do in aquatic vegetation, although always in areas with vegetation (Spence *et al*., 2006, 2008). As with *G. aculeatus*, this may reflect the lack of protection afforded by these types of shelter from predators, such as the Indian leaf fish *Nandus nandus* (Hamilton 1822) (Bass & Gerlai, 2008)), or may be due to the highly social nature of *D. rerio* (Al-Imari & Gerlai, 2008) which buffers or overrides any preference for shelter (Schroeder *et al*., 2014; White *et al*., 2017; Graham *et al*., 2018). Recent work showing that *D. rerio* prefer combinations of enrichment types over areas with a single form of enrichment (DePasquale *et al*., 2019) may also imply that the simple forms of shelter in our experiment were not complex enough to elicit a response. Alternatively, the *D. rerio* in our study, unlike the *G. aculeatus*, were captive-bred, had been used in a previous experiment (Jones *et al*., 2018) and may behave differently to wild fish, particularly in response to stressors such as handling and movement between tanks (Huntingford, 2004). Moreover, captive-bred fish may be less affected by standard laboratory lighting than wild-caught fish, since they were raised with it (Villamizar *et al*., 2014), although it can still be a major source of stress (Morgan & Tromborg, 2007; Lidster *et al*., 2017). While our study found no apparent value to either form of shelter enrichment, even indicating that *D. rerio* may actively avoid shade or plants, it was relatively short-term; longer periods of exposure to enrichment (gravel–plants) in *D. rerio* do show benefits in terms of both survivorship and female body condition (Lee *et al*., 2019). Additionally, it should be noted that it may take days for these species to fully acclimatise to a novel physical or social environment (typically *D. rerio* studies use up to a week prior to experimentation; Reolon *et al*., 2018) and that our experiments used small group sizes. Our results, therefore, may reflect the behaviour of mildly stressed individuals rather than those fully settled in their environment. We believe that this is, however, relevant to man laboratory contexts, where fish may be caught and transferred between treatments, conditions and group sizes on a regular basis.

Using choice tests, we have shown that while *D. rerio* show no apparent preference for shelter over open tank areas, *G. aculeatus* prefer both shaded and plant-containing areas over open areas, with a bias towards shade if given a choice between shelter types. Our study cannot provide information on more complex forms or combinations of physical enrichment for fish species, but instead emphasises the value of basic and convenient shelters such as a partial tank cover. These are low-cost and simple enough that they have the potential to become standardised aspects of fish welfare in laboratory conditions. The differences in preference between *D. rerio* and *G. aculeatus* highlights the importance of species-specific considerations in developing welfare protocols.

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Contributions

All authors contributed to the manuscript preparation and design of the experiment. N.A.R.J. conceived the idea, N.A.R.J., H.C.S.-J. and F.M.J. conducted the experiments, N.A.R.J. and H.C.S.-J. conducted the data analysis.

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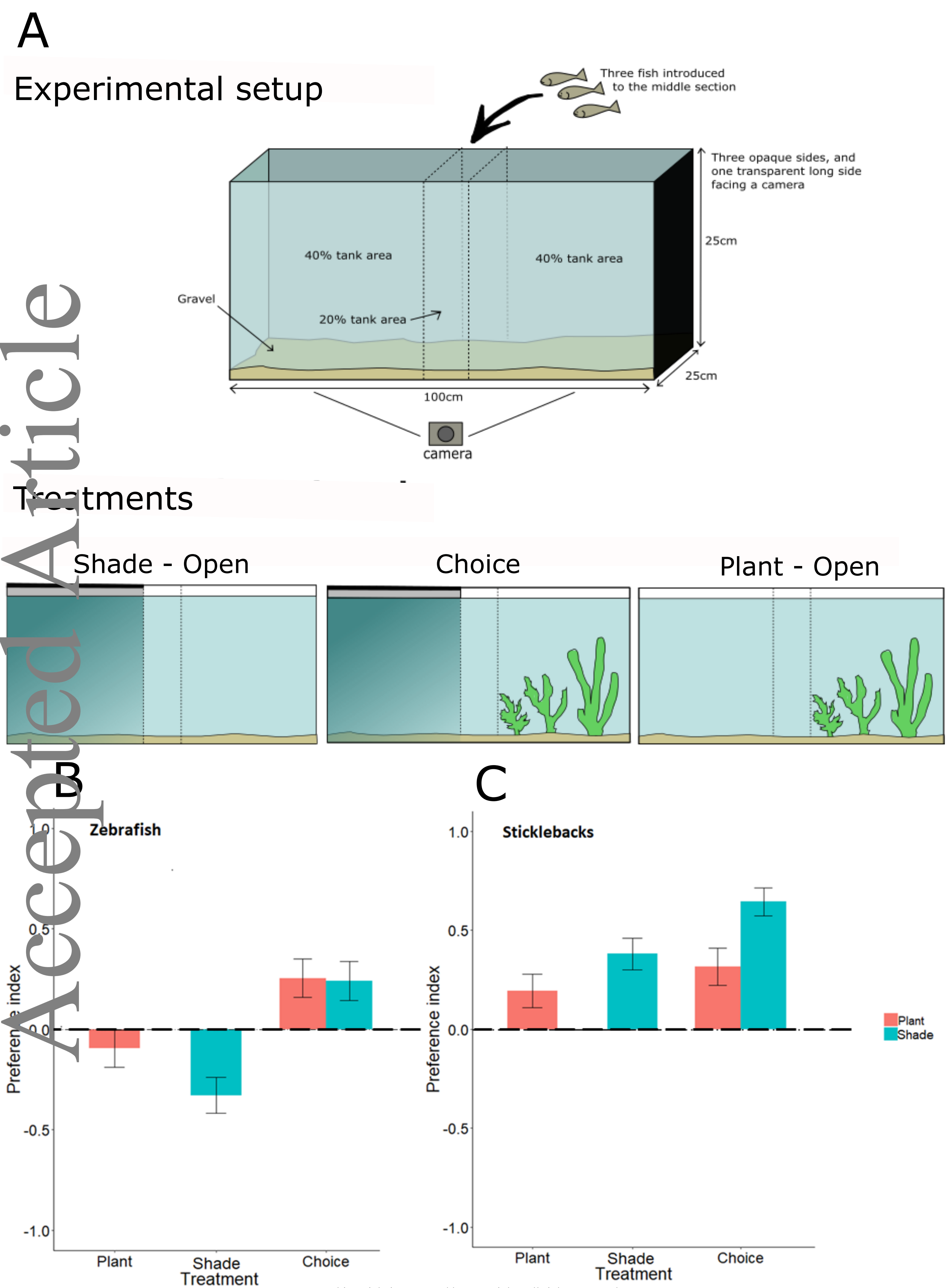
FIGURE 1 (a) Experimental setup and preferences for types of shelter (shade or open; shade or plants; plants or open) offered to zebrafish *Danio rerio* and sticklebacks *Gasterosteus aculeatus.* (Tank setup and conditions used in the experiment. (b) Mean (±95% CI) Jacobs preference index (*J*) for zebrafish and (c) sticklebacks when offered each of three focal shelter types. *J* = 1, strong preference; *J* = –1, strong avoidance.

Typesetter

1 Change A, B, C to (a), (b), (c).

2 Change all hyphens to en dashes


A
Experimental setup
Three fish introduced to the middle section
Three opaque sides, and one transparent long side facing a camera
25cm
40% tank area
40% tank area
Gravel
20% tank area
25cm
100cm
camera
Treatments
Shade - Open
Choice
Plant - Open
B
Zebrafish
1.0
0.5
0.0
-0.5
-1.0
Plant
Shade
Choice
Treatment
C
Sticklebacks
1.0
0.5
0.0
-0.5
-1.0
Preference index
Plant
Shade
Choice
Treatment
Plant
Shade