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Preference for structured environment in zebrafish (*Danio rerio*) and checker barbs (*Puntius oligolepis*)

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ABSTRACT

Information about the welfare and husbandry of pet and laboratory fish is scarce although millions of fish are sold in pet shops and used in laboratory research every year. Inadequate housing conditions can cause behavioural problems also in fish since they are complex animals with sophisticated behaviour. In this study, we investigated the influence of environmental complexity on compartment preference and behaviour in zebrafish (*Danio rerio*) and checker barbs (*Puntius oligolepis*). For the preference test, large aquaria were divided by two semi-transparent walls of Plexiglas into an empty compartment, a structured compartment enriched with plants and clay pots, and a smaller compartment in-between, where food was provided. For observation, the empty and structured compartments were divided into six zones of similar size by defining three vertical layers and two horizontal areas (back vs. front area). Seven groups of six to nine zebrafish and seven groups of seven or eight checker barbs were observed on four days each (within a time period of ten days) to assess compartment use and activity, and to assess behavioural diversity and use of zones within compartments. Both zebrafish and checker barbs showed a significant preference for the structured compartment. Nevertheless, in neither species did behavioural diversity differ between the empty and structured compartment. Zebrafish used all zones in both compartments to the same extent. Checker barbs, however, used the structured compartment more evenly than the empty compartment, where they mainly used the lower and middle zones. These results suggest that zebrafish and checker barbs have a preference for complex environments. Furthermore, they indicate that the behavioural and ecological needs of fish may vary depending on species, and recommendations for husbandry should be specified at species level.

1. Introduction

Millions of fish are produced for science, food industries and recreational activities every year (Huntingford et al., 2006; Saxby et al., 2010). While welfare concerns of mammals and birds have been discussed for several years and methods to assess welfare have been established (Hughes and Duncan, 1988; Broom, 1991; Mason and Mendl, 1993; Mendl, 2001; Dawkins, 2006; Boissy et al., 2007; Wechsler, 2007), in fish this process is only at the beginning (Chandroo et al., 2004; Huntingford et al., 2006; Ashley, 2007; Volpato, 2009). Since knowledge about fish behaviour and their skills, such as cognitive abilities (Bshary et al., 2002) or social learning (Brown and Laland, 2003), is accumulating, more and more scientists address the issue of welfare in fish (Broom, 2007). Studies on fish

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brains show that cognitive abilities, e.g. spatial cognition, are based on neural mechanism homologous to those of mammals and birds (Broglio et al., 2003). Huntingford et al. (2006) point out that fish are complex animals with sophisticated behaviour that are therefore likely to have the capacity to suffer. Others still deny that fish are sentient animals, but nevertheless advocate a respectful and responsible handling of fish (Rose, 2002; Iwama, 2007). However, as in mammals and birds (Mason et al., 2007), the well-being of fish can be compromised when housing conditions are not adequate (Ashley, 2007; Iwama, 2007). Inappropriate housing can cause chronic stress in fish (Huntingford et al., 2006). As a consequence, fish show disease symptoms, develop abnormal behaviours such as extended aggression or stereotypies, or become apathetic, e.g. bottom-sitting (Casamitjana, 2004; Ashley, 2007).

Ornamental fish have become increasingly popular pets over the last years and millions of fish are kept in house aquaria worldwide (Livengood and Chapman, 2007). In basic research and for testing chemicals, numbers of fish that are used as model organisms are also increasing (Johansen et al., 2006). In UK and Switzerland, fish have become the third most used experimental animals after mice and rats in research (BVET, 2009; Williams et al., 2009). Nevertheless, information about the welfare and husbandry in ornamental and laboratory fish is rather poor (Huntingford et al., 2006; Lawrence, 2007). In mammals and birds it has been shown that introducing environmental enrichment such as structural enrichment can create a stimulating environment that facilitates species specific behaviour, and behavioural problems may be reduced or even prevented (Shyne, 2006). Although structural enrichment such as plants, wood or different artificial structures are available to furnish aquaria, no information exists as to whether these structures are adequate and which structures are preferred by the numerous different ornamental fish species. Moreover, laboratory fish are usually held in small barren tanks what may cause behavioural problems, similar to laboratory mice kept in standard barren cages (Würbel et al., 1998). To date, only few studies on the effect of the physical surroundings have been conducted (e.g. Rotllant et al., 2003 in red porgy (*Pagrus pagrus*); Spence et al., 2007a in zebrafish (*Danio rerio*); Galhardo et al., 2008 in African cichlid (*Oreochromis mossambicus*); Barcellos et al., 2009 in silver catfish (*Rhamdia quelen*)).

In this study, we investigated the preference of two ornamental fish species, zebrafish (*Danio rerio,* Cyprinidae) and checker barbs (*Puntius oligolepis,* Cyprinidae) for structured environments. Zebrafish are often held in home aquaria, but more importantly they have become a vertebrate biomedical research model of paramount importance (Vascotto et al., 1997; Spence et al., 2008). Some characteristics such as high fecundity, small size, fast development and their supposedly simple husbandry requirements make this species attractive for laboratory researchers. In laboratories, zebrafish are normally held in small barren tanks (Spence et al., 2008 and pers. observation), a situation which does not reflect the natural conditions the fish are adapted to. In their natural range in India, Bangladesh and Nepal zebrafish occur in shallow water bodies with aquatic vegetation and silty substratum (McClure et al., 2006; Engeszer et al., 2007; Spence et al., 2008).

To investigate a further common pet species, we selected the checker barb or island barb (*Puntius oligolepis*, Cyprinidae) that is a typical form of the group of barbs. Barbs are small, group living freshwater fish with nice colours and various patterns, which renders them popular ornamental fish species for home aquaria in general. Moreover, they are considered to be easy to keep, although there exists only anecdotal information about their husbandry. According to the non-scientific aquarist literature, checker barbs naturally occur in Southeast Asia and live in cover-rich areas along the banks of brooks, rivers and lakes (Riehl and Baensch, 1983).

Based on both species' ecology they were selected as interesting case studies to examine their preference for structural enrichment in a choice experiment, and thus contribute to the question of adequate housing conditions in ornamental and lab fish.

The fish were offered the opportunity to choose between two compartments, one of which was structured with plants and clay pots while the other one was left empty. Between these two compartments there was a smaller compartment where food was offered. Preference tests are widely used in animal welfare research (Mason and Mendl, 1993) and may yield useful information about what animals want (Dawkins, 2003). We predicted that both checker barbs and zebrafish would spend more time in the structured compartment than in the empty compartment. Along with the more intense use of the structured compartment, we expected a higher diversity of the behavioural repertoire in the structured compartment. Furthermore, we expected that the fish use the space differently in the structured compartment because the structures can fulfil behavioural functions such as providing cover or hiding opportunities against aggressive conspecifics or other fish species, and make space more accessible to the fish by partitioning it; thus the fish would be able to move more safely and orientate themselves with the help of structures.

2. Materials and methods

2.1. Subjects and housing

The study was conducted in an indoor facility for animal housing. Fifty-two (21 females and 31 males) checker barbs (*Puntius oligolepis*) were obtained from a pet shop. They were subdivided in seven groups of seven (4 groups) or eight individuals (3 groups) of both sexes. The groups were placed in seven aquaria of similar, but somewhat variable size (Table 1). Of the 56 zebrafish (*Danio rerio*), 47 of the wild-type strains Tü, AB, and WIK, and albino were provided by the department of Neurobiology of the University of Zurich where they had been raised in standard tanks without structures. They had not participated in any other scientific study before. The other 9 zebrafish were obtained from a pet shop. The zebrafish were subdivided in seven groups of 6–9 individuals of both sexes: four mixed groups of Tü and WIK, one albino group, one AB group, and the pet shop group (Table 1). The sex of the zebrafish was

Table 1
Sizes of the seven aquaria used in our study (AQ), position of the structural enrichment, and sizes and composition of seven groups of zebrafish and checker barbs.

AQ	Size (lenght × width × height, cm)	Structured compartment	# Zebrafish	Zebrafish strains	# Checker Barbs
1	130 × 50 × 50	Right	8	Tü + WIK	8
2	100 × 50 × 50	Left	6	Albino	7
3	100 × 50 × 50	Left	8	Tü + WIK	7
4	130 × 50 × 50	Right	8	Tü + WIK	8
5	160 × 41 × 50	Left	9	AB	7
6	130 × 50 × 50	Right	8	Tü + WIK	7
7	100 × 50 × 50	Left	9	Pet shop	8

not defined because the differentiation between males and females was not possible for this species from the required observational distance. The zebrafish were held in the same tanks as the checker barbs after the checker barbs had been removed and the tanks thoroughly cleaned.

Each aquarium was equipped with a layer of sand of 2 cm, two internal filters (Eheim Aquaball, EHEIM GmbH & Co. KG, Germany), a heating element, plants (*Ceratopteris thalictroides*) and clay pots for cover. Water temperature was 25 (±1) °C and the light:dark cycle 12 h:12 h (lights on at 08.00 hours). To control water quality, 1/3 of the water in the tanks was changed weekly and checked for pH (7.0). Food was provided by automatic feeders (EHEIM 3581) several times per day and consisted of flake food.

2.2. Experimental set-up

Each tank was subdivided into three compartments (left, right and middle compartment; from the point of view of the observer) by two semi-transparent walls of Plexiglas (Fig. 1). A small hole in each wall permitted the fish to switch between compartments. With checker barbs, the walls were installed such that the holes were at the bottom of the wall, with zebrafish the holes were at the top of the walls. Pilot studies had shown that the fish learned these positions quickest. The left and the right compartment were both of the same dimensions though the equipment was varied across the seven tanks: In four tanks plants and clay pots were placed in the left compartment, and in three tanks plants and clay pots were placed in the right compartment. The other compartment was left empty except for the layer of sand (Table 1; Fig. 1). The middle compartment was smaller (distance between the walls: 30 cm) and equipped with two internal filters, the heating element, and the automatic feeder on top of the tank. The fish were provided with food in the middle compartment only, thus provoking the fish to actively choose between the structured and the empty compartment after feedings. The automatic feeders were adjusted such that only a small portion of flake food was released per feeding bout over four feeding bouts per day.

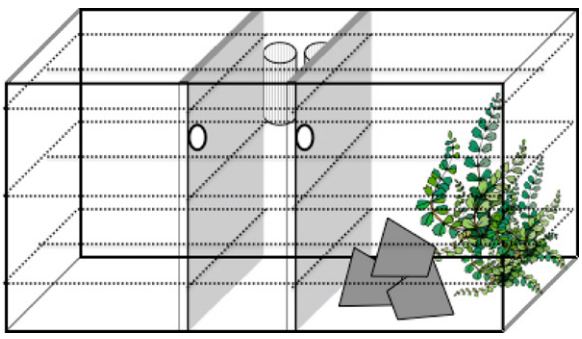

Fig. 1. Design of the choice experiment. The aquarium was divided by two semitransparent plates into three compartments, the empty compartment (left), the structured compartment (right), and the food compartment (middle). The holes permitted the fish to change between the compartments. The structured compartment was supplied with plants and clay pots. The middle compartment contained two internal filters. The dashed lines illustrate the virtual sectors used for behavioural observations.

2.3. Data recording

After their arrival from the pet shop, checker barbs were habituated to the experimental setup for ten days. After the transfer from the Neurobiology lab, the four strains of zebrafish were held in an extra tank (100 cm × 40 cm × 50 cm) that was partitioned in four equally sized compartments and equipped with plants and clay pots for 18 days to check for health or behavioural problems. Thereafter, they were transferred to the experimental tanks and habituated to the experimental setup for eight days. Also the pet shop group was habituated to the experimental setup for eight days.

All data were recorded on four days within a seven-day period. To assess compartment use, the position of all fish was recorded four times a day between 10.00 hours and 17.00 hours: two recordings 30 min after a feeding event in the morning (10.30 hours) and in the evening (16.30 hours), and two recordings 15 min after a feeding event at noon and in the early afternoon (14.00 hours).

To quantify activity, the number of switches (of any fish) from the middle compartment to the left and right compartment was recorded for 10 min after the feeding events at noon and in the early afternoon. This measure was taken to check if the fish were actually switching between the compartments. For feeding, the fish had to swim into the middle compartment. Therefore, we assumed that the choice of a compartment after feeding could be considered as an independent option.

Data to assess behavioural diversity were collected using instantaneous observations at 5 min intervals. Behaviour was recorded for 20 min, once in the morning and once in the afternoon. All behaviours were classified into one of six categories of functionally related behaviour (Table 2): exploration, foraging, social behaviour (socio-positive and socio-negative), resting, comfort behaviour,

Table 2
Behavioural categories and description of behavioural elements.

Behavioural category	Behavioural element	Description
Exploration	Swimming	Slow displacement of the body through the water with body undulation and fins movements
	Investigating	Muzzle close to structures such as plants and clay pots
	Floating	Very slow displacement through the water, hardly moving the fins
Foraging	Feeding	Ingesting food items
	Dabbling	Investigation of the sand layer, head pointing downwards, tail fin pointing upwards
	Picking plants	Fish feeds on plant leaves
	Rasping	Fish feeds on algae from the glass pane
Resting	Inactive	Fish remains motionless
Locomotion	Fast swimming	Displacement at high speed
Socio-positive behaviour	Group swimming	Fish changes position together with a group of fish consisting of at least three individuals
	Following	Fish swims close (max one body length of distance) behind one conspecific in the same direction (no third conspecific is involved), fins in normal position
	Approaching	Fish swims directly towards a conspecific to a distance of less than one body size
Socio-negative behaviour	Threatening	Fish stands closely (max one body length) parallel or anti-parallel to a conspecific, fins are raised
	Attacking	Fish moves towards a conspecific at high speed and conspecific moves away
	Defending	Fish chases a conspecific away from a structure
	Escaping	Fish moves away from an attacking conspecific
Mating behaviour	Paralleling	Fish is close aside a conspecific moving in the same direction, fins are raised, no third individual involved
	Swimming ahead	Fish moving away from a conspecific that shows raised fins
	Pursuing	Fish follows a conspecific with raised fins
Comfort behaviour	Rubbing	One side of the body touches the ground
"Stereotypy"	Waving	Repetitive movement, fish swims to an fro of the front glass pane

locomotion (other than exploring and foraging), and waving. Waving describes a repetitive movement: the fish swims to and fro in front of the front glass pane. It might be the same behaviour that is described as pacing which is classified as a stereotypy (Casamitjana, 2004). All aquaria were scanned starting from the left compartment going to the right compartment, and in each compartment the behaviour of the second fish that was detected was recorded. If there was only one fish in the compartment, the behaviour of this fish was recorded, and if there was no fish, the scan was left empty.

Along with the behaviour, the position of all fish in each compartment was recorded to evaluate space use (localisation of fish was always possible). For this purpose, the aquarium was virtually divided vertically into three layers (lower layer, middle layer, upper layer) and horizontally into two zones (back and front), thus creating six zones of equal size: low front, low back, middle front, middle back, upper front, upper back; front referred to the section closest to the observer. All data were collected by direct observations. The fish were used to the observer's presence in front of the tanks, as they showed no fear or flight reaction and were not attracted to the front while recording data.

2.4. Data analysis

For the analysis of the preference for either the structured or empty compartment, we used data of seven groups of each species. For the analysis of behavioural data, we included data of six groups of each species only. One group of each species had to be excluded due to constraints in daily observation time.

To quantify the preference for either the structured or empty compartment, the percentage of fish per compartment and aquarium was calculated for each sampling point (16 in total). In some cases, most of the fish were in the middle compartment and showed no selection for either the structured or the empty compartment. Therefore, only when three or more fish were observed outside the middle compartment, data were included in the analysis. Based on these percentages, a mean value per aquarium was calculated. In order to obtain a preference score for structure use, the Jacobs' preference index (Jacobs, 1974) was calculated as

$$J = \frac{(r - p)}{[(r + p) - 2rp]}$$

where r is the ratio of the number of fish in the structured compartment to the number of fish in the structured compartment plus the number of fish in the empty compartment, and p is the available proportion of the empty and the structured compartment of the experimental space in the aquarium, respectively, in this case $p = 0.5$. The index ranges between +1 for maximum preference, and −1 for maximum avoidance. To examine preference for the structured compartment over the whole observations period (16 sampling points) the index was calculated per aquarium. To test for non-random use of structures (significant difference from zero) a one-sample t-test was conducted (with $n - 1$ degrees of freedom, n is the number of aquaria in the analysis).

To determine activity, we calculated a switch rate r_{ch} during the observation period (8×10 min), where r_{ch} is the number of changes from the middle compartment to the left compartment plus the number of changes from the middle compartment to the right compartment divided by the number of individuals in the tank. Based on these rates, a mean switch rate r_{ch} per species was calculated.

For the activity budget, the percentage of each behavioural category was calculated in both compartments. As social behaviour might be particularly influenced by structural enrichment (Basquill and Grant, 1998; Carfagnini et al., 2009), social behaviour was further divided in the following sub-categories socio-negative, socio-positive and courtship behaviour. These sub-categories were analysed using the Wilcoxon matched-pairs signed-rank test (Zar, 1999) to determine the differences between the empty and the structured compartment.

To quantify behavioural diversity, the number of behaviour patterns per behavioural category was summed up per compartment over the total observation time. Based on these numbers, the Shannon index of diversity H (Shannon and Weaver, 1949) was calculated as

$$H = -\Sigma(p_i \ln p_i),$$

where p_i is the relative abundance of each behavioural category, calculated as the proportion of behavioural elements of a given category to the total number of behavioural elements of all categories: n_i/N. The index was calculated per aquarium. It increases with increasing numbers of behavioural categories, and as the relative representation of each category becomes more even. Lower indices represent lower behavioural diversity. The Wilcoxon matched-pairs signed-rank test was used to determine the differences between the empty and the structured compartment.

To quantify space use, the number of visits per zone over the total observation time was summed up. Based on these numbers, the spread of participation index (SPI) developed by Dickens (1955) was calculated as

$$\mathrm{SPI} = \frac{M[(n_b - n_a)/(F_a - F_b)]}{2(N - M)}$$

where N is the total number of observations in all zones, M the mean frequency of observations per zone (M/N), n_a the number of zones with observations > M, n_b the number of zones with observations < M, F_a the total number of observations with observations > M, and F_b the total number of observations with observations < M. The index was calculated per structured and empty compartment and per aquarium. An SPI value of 1 indicates minimum utilisation, i.e. the fish would spend all their time in one zone; a value of 0 indicates maximum use, i.e. the fish would use all zones equally. The Wilcoxon matched-pairs signed-rank test (Zar, 1999) was used to determine the differences between the empty and the structured compartment.

SPSS (Version 18.0 for Windows) was used for all statistical tests.

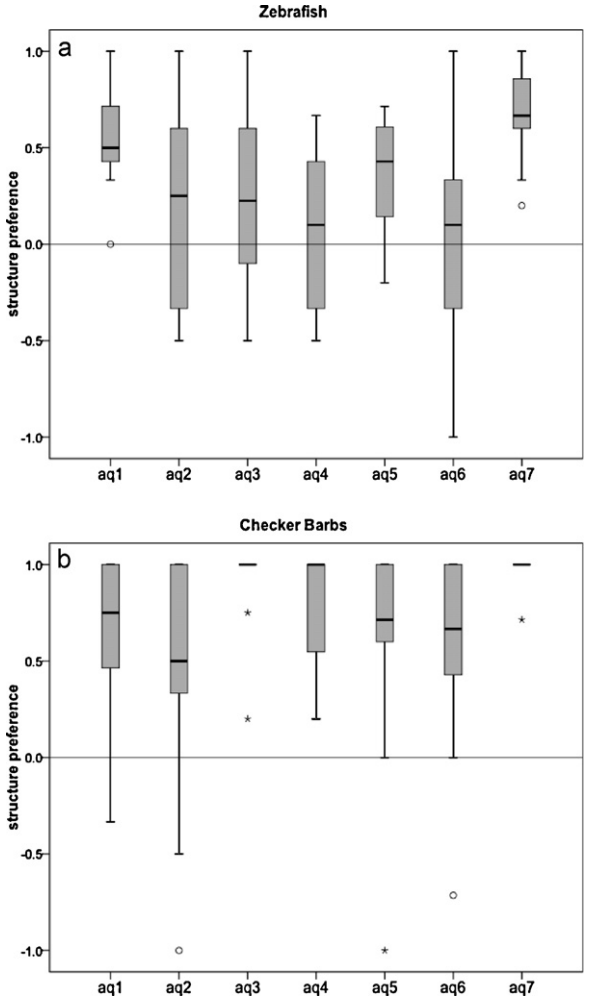

Fig. 2. Jacobs' preference index for the use of the empty and the structured compartment in seven aquaria for (a) zebrafish and (b) checker barbs. Positive and negative values indicate preference and avoidance, respectively; boxes indicate the 25 ± 75th percentile range and contain the median line; bars represent the 10th and 90th percentile values; open dots represent points outside these values.

3. Results

3.1. Compartment preference

In zebrafish, mean use of the three compartments was 21%, 35% and 44% for the empty, middle and structured compartments, respectively. Over all seven tanks, the zebrafish showed a significant preference for the structured compartment (Jacobs' preference index: $t = 3.41$, df = 6, $p = 0.01$; Fig. 2a). The checker barbs also showed a significant preference for the structured compartment ($t = 9.56$, df = 6, $p = 0.0$; Fig. 2b), with a mean use of 9%, 28% and 63% for the empty, middle and structured compartments, respectively. In zebrafish the mean switch rate r_{ch} per 10 min between the middle compartment and the empty was 1.30 (±0.09), and between the middle and the structured compartment 1.86 (±0.12). For checker barbs the respective values were 0.43 (±0.05; middle to the

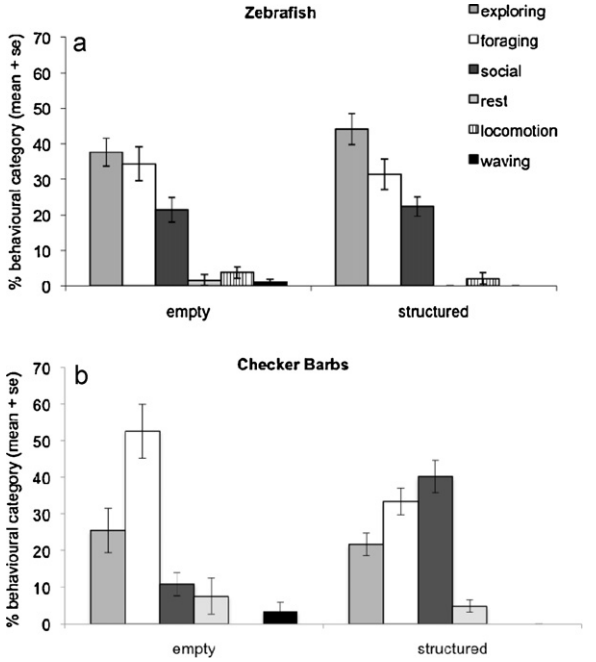

Fig. 3. Activity budget (overall mean + SE) for (a) zebrafish ($n = 6$ aquaria) and (b) checker barbs ($n = 6$).

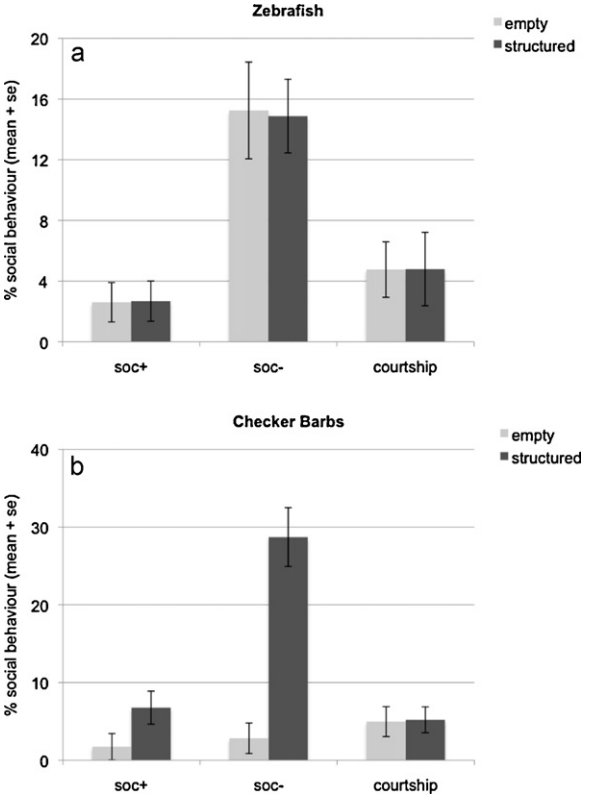

Fig. 4. Percentage (mean + SE) of socio-negative, socio-positive (soc+) and mating (courtship) behaviour in (a) zebrafish ($n = 6$) and (b) checker barbs ($n = 6$).

empty compartment), and 1.18 (±0.09; middle to the structured compartment).

3.2. Behavioural diversity and compartment use

Fig. 3a and b show the activity budgets of the zebrafish and checker barbs. Both in the empty and structured compartment, zebrafish showed similar amounts of exploration, foraging and social behaviour. Checker barbs instead showed high levels of foraging in the empty compartment, in contrast to high levels of social behaviour in the structured compartment. In both species, waving was observed in the empty compartment only.

Fig. 4a and b show the percentage of social behaviour (socio-positive, socio-negative, and mating behaviour) in the structured and in the empty compartment. In zebrafish, the occurrence of socio-negative, socio-positive and courtship (mating) behaviour did not differ significantly between the empty and the structured compartment (Fig. 4a). In the structured compartment, checker barbs showed significantly more socio-negative behaviour ($Z = -2.201$, $p = 0.028$, $n = 6$; Fig. 4b), and tended also to show more socio-positive behaviour ($Z = -1.753$, $p = 0.08$, $n = 6$; Fig. 4b), but showed no significant difference in courtship behaviour between the two compartments (Fig. 4b).

Diversity of behaviour did not differ significantly between the empty and structured compartments, neither in zebrafish ($Z = -0.943$, $p = 0.345$, $n = 6$; Fig. 5a), nor in checker barbs ($Z = -1.572$, $p = 0.116$, $n = 6$; Fig. 5b).

In both compartments, the zebrafish used the front zones more often than the back zones (Fig. 6a). In the empty compartment, peak use was observed in the upper front zone, while the lower and middle zones in the back and front were used to similar extents, resulting in a SPI_{empty} of 0.43. In the structured compartment, the zones were used more evenly as indicated by a slightly lower $SPI_{strucuted}$ of 0.37. There was no significant difference between the SPIs of the empty and the structured compartments ($Z = -0.734$, $p = 0.436$, $n = 6$).

Checker barbs used the structured compartment more evenly than the empty compartment, as indicated by a significantly lower SPI in the structured compared to the empty compartment ($SPI_{strucuted} = 0.46$, $SPI_{empty} = 0.62$; $Z = -1.992$, $p = 0.046$, $n = 6$). In the empty compartment, the checker barbs used lower zones to a high degree (Fig. 6b). In the front of the structured compartment, the checker barbs were observed more in the lower zone, whereas in the back they were observed more in the middle zone. In both compartments, the checker barbs were rarely observed in the upper zones (Fig. 6b).

4. Discussion

4.1. Structure preference

Over all seven groups, and irrespective of strain, zebrafish showed a significant preference for the structured over the empty compartment. Although the strains of zebrafish used in this study have been bred and kept without structures for generations, the preference for structures

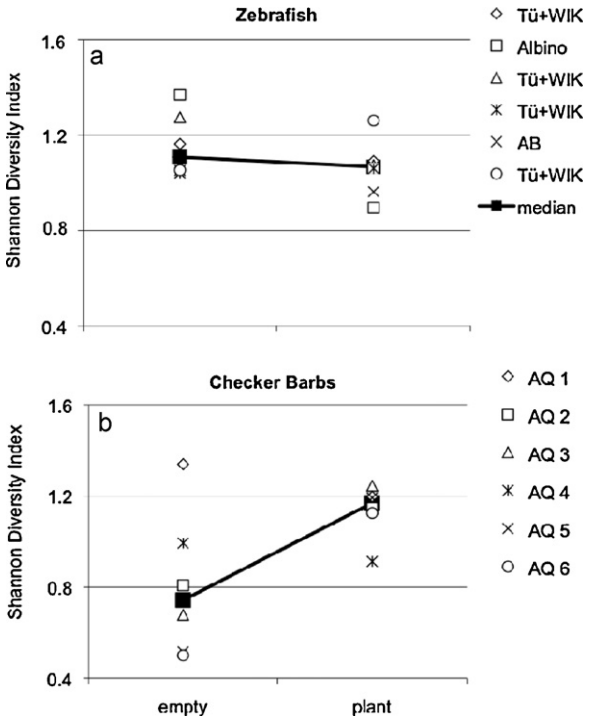

Fig. 5. Behavioural diversity in the empty and the structured compartment (plant and pots) for (a) zebrafish and (b) checker barbs. Values of Shannon diversity index of six aquaria and overall median are shown. Lower index values represent lower behavioural diversity, and higher values represent higher behavioural diversity.

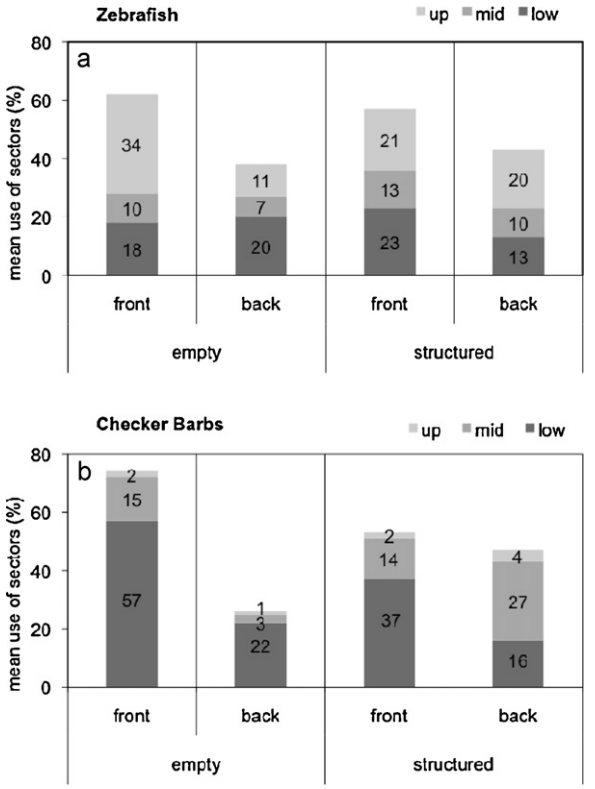

Fig. 6. Mean use of sectors in the empty and the structured compartment for (a) zebrafish ($n = 6$) and (b) checker barbs ($n = 6$). The six sectors are: upper front, upper back, middle front, middle back, lower front, and lower back.

was very pronounced. This suggests that this preference may be the result of selection under natural conditions. Our results are in line with findings from field studies where wild zebrafish were found in well-vegetated shallow water bodies (Spence et al., 2006; Engeszer et al., 2007). Structural enrichment such as plants or clay pots can fulfil different functions in aquaria, such as substrates for oviposition, cover or food. In a study on preference for substrates, domesticated zebrafish showed a preference for vegetation for oviposition, but wild caught zebrafish did not show such a preference (Spence et al., 2007a). Zebrafish in the wild, however, were observed to deposit eggs in shallow areas with vegetation where they are protected from predators (Spence et al., 2006; Engeszer et al., 2007). In their study on zebrafish in the wild, Engeszer et al. (2007) mention a number of predator species that feed on zebrafish of various developmental stages. In captive zebrafish, predator avoidance might not be of high importance, however, areas with overhead cover are used more often than open areas suggesting that zebrafish aim to avoid predation risk (Hamilton and Dill, 2002). Moreover, adult zebrafish prey on zebrafish eggs and larvae, and it is assumed that zebrafish larvae need plants to survive because they help them to reach the water surface (Spence et al., 2008). Structures can also serve as optical barriers and provide protection from disturbances or from conspecifics (Williams et al., 2009).

Checker barbs also showed a significant preference for the structured compartment. This confirms the information from the non-scientific aquarist literature where plants are recommended for structuring (Riehl and Baensch, 1983). No information is available about predators of wild checker barbs. However, as checker barbs are about the same size as zebrafish, it can be assumed that various predators also feed on checker barbs. Observations from captive checker barbs revealed that they use plants to deposit eggs (Riehl and Baensch, 1983). Therefore, in zebrafish and checker barbs structural enrichment such as plants and clay pots probably fulfils multiple functions such as providing spawning sites, shelter or division of space.

4.2. Behavioural diversity and sector use

Zebrafish displayed similar behavioural diversity in both compartments. In the empty compartment, behaviour was slightly more evenly distributed among the six behavioural categories, resulting in a higher median diversity index. In both compartments, zebrafish showed high levels of dabbling (i.e. searching for food in the sand) and swimming, whereas investigating was observed more frequently in the structured compartment, a behaviour that was also directed to clay pots. Rasping algae from the glass pane was shown more frequently in the empty compartment but to a similar extent as picking plants in the structured compartment. In both compartments, zebrafish showed similar amounts of socio-positive behaviour and

socio-negative behaviour, respectively. The partition of the aquaria was probably used to avoid aggressive conspecifics as zebrafish often switched between the compartments. Increased aggressive behaviour can be a welfare issue (Galhardo et al., 2008), however, in relation to territorial behaviour aggression forms part of the natural behaviour. In our study we used large aquaria, thus individuals could avoid each other, and no signs of stress such as change of colour, apathetic behaviour or health problems (Casamitjana, 2004) were observed. Structural enrichment was shown to reduce aggressive behaviour and monopolisation of food in zebrafish (Basquill and Grant, 1998; Carfagnini et al., 2009). Aggressive behaviour is associated with dominance in males and females, and dominance is supposed to play a role in reproductive behaviour and in maintaining the social structure in zebrafish (Paull et al., 2010). As we did not distinguish between individuals and sex, information about dominance hierarchy was not available. In our study, food was provided in a separate compartment; therefore we assume that in the empty and the structured compartment the observed aggressive behaviours such as chasing or defending were mainly related to reproductive behaviour. Wild male zebrafish engage in both territoriality and active pursuit of females and defend potential spawning sites (Spence et al., 2007a; Hutter et al., 2010), but also females engage in aggressive behaviour (Paull et al., 2010). The comparable level of aggressive behaviour suggests that zebrafish monopolised in both compartments potential spawning sites, although these sites varied in quality (Spence et al., 2007a).

Also in checker barbs there was no significant difference between the empty and the structured compartment regarding behavioural diversity. However, all groups showed the highest absolute numbers of behavioural elements in all behavioural categories in the structured compartment. In the empty compartment, checker barbs showed more dabbling and rasping algae from the glass pane (foraging behaviour) than in the structured compartment where they were frequently observed picking plants (foraging behaviour). In the structured compartment they exhibited a higher amount of both socio-negative and socio-positive behaviour than in the empty compartment. Particularly male checker barbs displayed socio-negative behaviours such as defending, chasing, and threatening. According to the non-scientific aquarist literature male checker barbs often show threatening behaviour, but usually do not fight (Riehl and Baensch, 1983), and it is supposed that they are territorial and defend spawning sites (Kortmulder, 1981). In our study it seemed that structures promoted territorial behaviour as aggressive behaviour occurred more often in the structured compartment.

Overall, although there was no difference in behavioural diversity between the compartments in both species, structural enrichment seems to play an important role in social behaviour. Moreover, foraging behaviour was directed to the substrate or to the glass panes in the compartments in which structures were lacking, and waving as a potential stereotypy was observed.

Regarding space use in zebrafish, there was neither a significant difference between the empty and the structured compartment, nor a significant preference of a zone. However, the distribution of zone use was slightly more balanced in the structured compartment; in the empty compartment, the zebrafish spent much time in the upper front zone, possibly due to the opening that was positioned in the upper front part of the wall. Behavioural observations and diet analysis revealed that zebrafish occupy the whole water column and also feed on food items on the water surface (Spence et al., 2006, 2007b). Flake food provided by the feeder on top of the aquaria was mostly consumed at the surface. This may be a reason why the zebrafish spent a considerable proportion of time in the upper zones in both the empty and the structured compartment.

Checker barbs used the zones in the structured compartment more evenly than in the empty compartment. In the structured compartment, the checker barbs spent most time in the lower and middle zones. In the empty compartment, they used the lower zones to a high degree, preferably the lower front zone. The strong use of the front sectors in both compartments could have occurred because the openings at the lower end of the walls allowed the fish to quickly switch between the compartments. However, in the structured compartment they also used the middle sector in the back to a high degree where plants were present. And although plants were also present in the upper sector of the structured compartment, the fish avoided this sector. Altogether, the results indicate that structuring the aquarium makes the space more accessible to checker barbs, and that they orientate their activity preferably to the lower and middle levels of the aquarium.

Simple choice tests have their limitations, since preferences may depend on context or experience (Kirkden and Pajor, 2006). The zebrafish of the pet shop group differed from the laboratory strains in their origin and therefore experienced other environmental conditions during their development. Although all groups preferred the more complex environment, the pet shop group showed the most pronounced preference. This group probably had prior experience with structures at least in the pet store, but no information was available about rearing conditions. Pet and laboratory fish have been bred in captivity for generations and are therefore domesticated to some extent (Balon, 2004). However, the animals' behavioural organisation was shaped by the environmental conditions of their natural habitats, and checker barbs and zebrafish originate from structured environments (Riehl and Baensch, 1983; Spence et al., 2006). Considering the results of the two species, it seems that checker barbs are more bound to structures than zebrafish, indicating differences in behaviour and use of the natural habitat. Early experience and rearing conditions but also domestication processes might have influenced the extent of the preference. However, it has been shown that domestication and intensive housing have hardly changed the behavioural repertoire of farm animals (e.g. in pigs; Stolba and Woodgush, 1989), and therefore the same may be true for environmental preferences. In future studies, more sophisticated preference tests using measures of strength of preference or changing preference in the presence of further resources (Kirkden and Pajor, 2006), or physiological measures such as stress hormones (Mendl,

2001) should be examined. However, simple choice tests as used here are thought to yield valid information about what animals want (Dawkins, 2003) and are a first step into examining species-specific housing conditions for ornamental fish used as pets or laboratory animals.

Finally, performing preference tests on a group level might have caused group dynamic effects such as dominance of individuals over others. However, both zebrafish and checker barbs are naturally group living fish and may perform better in groups (Riehl and Baensch, 1983; Spence et al., 2008). Moreover, keeping them solitary would have most likely caused a frightening and stressful situation, which could have severely influenced their behaviour.

4.3. Concluding remarks

There is a huge variety of fish species that are kept in home aquaria and requirements for species adequate housing differ considerably (Livengood and Chapman, 2007). When enclosures (or aquaria) lack critical resources and stimuli that facilitate species-typical behaviour, behavioural disturbances can arise (Mason, 1991; Casamitjana, 2004). Interestingly, in our study, waving, a repetitive movement in front of the glass pane, occurred in both species only in the empty compartment. Waving could represent the same behaviour as pacing (continuous swimming to and fro) which has been classified as stereotypy in fish (Casamitjana, 2004). This could indicate that a barren environment, typical for laboratories, can cause behavioural problems. Considering the complex behaviour, physiology and brain anatomy of fish, it is likely that also fish may suffer from inadequate housing conditions. It has been shown across different taxa that the behaviour of captive animals can be influenced by adding structural heterogeneity to the environment, and that their well-being may be improved by an enriched environment (Balcombe, 2006; Mason et al., 2007; Kistler et al., 2010). However, structural enrichment needs to be adjusted to the behavioural and ecological needs of a species as structural enrichment can fulfil different functions such as providing cover, food or potential spawning sites in fish. In our study, zebrafish and checker barbs showed a clear preference for structures, but they used the water column differently. Foraging strategies and mating tactics probably influenced the use of space in both species. Our results from a simple choice test suggest that structural complexity in aquaria and its species-specific arrangement may be beneficial for the well-being of zebrafish and checker barbs.

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