The Importance of Environmental Enrichment for Explorative Animals in Captivity - Red Foxes (*Vulpes vulpes*) and Cyprinid Fish as Case Studies

Claudia Kistler

von

Zürich und Reichenburg (SZ)

The Importance of Environmental Enrichment for Explorative Animals in Captivity - Red Foxes (*Vulpes vulpes*) and Cyprinid Fish as Case Studies

Claudia Kistler

CONTENTS

SUMMARY

Animal welfare science is concerned about the welfare of millions of animals living in captivity. To improve housing conditions, environmental enrichment is used to provide specific environmental stimuli, and encompasses feeding, structural, social or olfactory enrichment. Adding complexity to the housing environment proved to reduce behavioural disturbances such as stereotypies in many studies. Therefore, it is necessary to experimentally identify environmental stimuli that allow species-specific behaviour.

In my thesis, I adopted a naturalistic approach, by investigating animals under naturalistic conditions and providing them with stimulating environments (or naturalistic stimuli) that are based on the natural habitat and the natural behaviour of the studied species. Furthermore, I used a comparative approach to verify the general principle of using naturalistic stimuli to promote species-typical behaviour. In particular, I was interested in how environmental enrichment influences the behavioural diversity and space use. In an outdoor test enclosure, I investigated the effect of a feeding and a structural enrichment in a group of red foxes (*Vulpes vulpes*). After their transfer to a newly built, natural-looking outdoor enclosure, I investigated their structure use. Furthermore, I investigated different barbs species (*Puntius* spp.) and zebrafish (*Danio rerio*) in a choice experiment providing an empty and a structured environment. Preference tests are a widely used tool to reach valid information about what animals want. Red foxes and cyprinids are both highly explorative animals feeding opportunistically on a wide trophic niche, but also experience high predation, and both taxa thus should respond to (and profit) from environmental enrichment in captivity.

The foxes were presented with a varied feeding enrichment using automatic devices and different food items that simulated a natural situation. Conventional feedings usually offer food concentrated in space and time. I found that a feeding enrichment based on the animals' natural activity patterns and foraging strategies stimulates species-specific behaviours. In particular, for species that suffer high predation risk and whose natural habitats are cover-rich, cover may also be an important feature to allow the animals to range throughout the enclosure in captivity. The foxes preferred to range along long structures and through small cover structures imitating hedges and thickets, respectively. These findings were confirmed in the newly built enclosure into which the foxes were transferred and where they also showed a pronounced preference for areas containing structures. The structures were arranged net-like throughout the enclosure, and thus the foxes were able to retreat or move along a nearby structure while ranging. In the choice experiment, all but one barb species and the zebrafish preferred a structured to a barren environment, but the type of environment did not influence behavioural diversity, probably due to feeding occasions and the variable social behaviour. I also found that different strains of zebrafish showed all a preference for a structured environment. Interestingly, checker barbs and zebrafish showed waving, a stereotypic-like behaviour, only in the barren environment. Furthermore, checker barbs and zebrafish used the water column differently, indicating species-specific differences. Thus, I found both in foxes and the fish a preference for structural enrichment simulating the natural habitat.

This thesis showed that the concept of environmental enrichment, i.e. experimentally identifying environmental stimuli to establish species-adequate housing and prevent behavioural disturbances, applies for a wide range of vertebrate taxa. The findings support the hypothesis that

stimulating environments are important for the normal brain development and therefore normal behaviour.

ZUSAMMENFASSUNG

Die Tierschutzforschung beschäftigt sich mit dem Wohlbefinden von Millionen von Tieren, die in Gefangenschaft leben. Um die Haltungsbedingungen zu verbessern, werden mittels der Methode der Umweltanreicherung (Environmental Enrichment) spezifische Umweltreize angeboten. Environmental Enrichment umfasst verschiedene Bereiche, wie Fütterungsenrichment, strukturelles, soziales oder olfaktorisches Enrichment. In vielen Studien wurde gezeigt, dass zusätzliche Komplexität in der Haltungsumgebung Verhaltensstörungen wie zum Beispiel Stereotypien reduzieren kann. Daher ist es wichtig, experimentell die Umweltreize zu identifizieren, die ein artspezifisches Verhalten auslösen.

In meiner Dissertation habe ich einen naturalistischen Ansatz gewählt, d.h. ich habe die Tiere unter natürlichen Bedingungen beobachtet und ihnen stimulierende Umweltbedingungen (oder natürliche Reize) angeboten, die auf dem natürlichen Habitat und dem natürlichen Verhalten der untersuchten Art basieren. Weiter habe ich einen vergleichenden Ansatz gewählt, um das allgemeine Prinzip zu verifizieren, dass natürliche Reize artspezifisches Verhalten fördern. Speziell war ich daran interessiert, wie Environmental Enrichment die Verhaltensdiversität und die Raumnutzung beeinflusst. In einem Aussentestgehege untersuchte ich die Auswirkungen eines Fütterungsenrichments und eines Strukturenrichments bei einer Gruppe von Rotfüchsen (*Vulpes vulpes*). Nachdem die Füchse in ein neu gebautes, naturnahes Gehege transferiert wurden, habe ich dort ihre Strukturnutzung untersucht. Weiter habe ich in einem Wahlexperiment verschiedene Barbenarten (*Puntius* spp.) und Zebrafische (*Danio rerio*) untersucht, denen ich gleichzeitig eine strukturlose und eine strukturierte Umgebung anbot. Präferenztests werden häufig genutzt, um herauszufinden, was Tiere wollen. Rotfüchse und Cypriniden sind sehr explorative Arten, ernähren sich opportunistisch, unterliegen aber auch einem hohen Räuberdruck. Beide Taxa sollten also auf das Environmental Enrichment reagieren (und davon profiteren).

Den Füchsen bot ich ein vielfältiges Fütterungsenrichment mit Fütterungsapparaten und unterschiedlichem Futter an, um die Situation im Freiland zu simulieren. Konventionelle Fütterungsregimes bieten das Futter häufig zur selben Zeit am selben Ort an. Ich konnte zeigen, dass ein Fütterungsenrichment, das auf den natürlichen Aktivitätsmustern und Futtersuchstrategien basiert, artspezifisches Verhalten stimuliert. In Gefangenschaft ist Deckung speziell bei Tierarten wichtig, die einem hohen Räuberdruck unterliegen und deren natürliche Habitate strukturreich sind. Deckung ermöglicht es ihnen, sich sicher durchs Gehege zu bewegen. Die Füchse zogen es vor, sich entlang von langen Strukturen und unter Deckungsstrukturen zu bewegen, die Hecken bzw. Gebüsche imitierten. Dieses Resultat bestätigte sich im neuen Gehege, wo die Füchse auch eine ausgeprägte Präferenz für strukturierte Areale zeigten. Die Strukturen waren netzartig im Gehege angeordnet, so dass sich die Füchse immer zurückziehen oder sich entlang einer Struktur bewegen konnten. Im Wahlexperiment zeigten die Barbenarten (mit einer Ausnahme) und die Zebrafische eine Präferenz für die strukturierte Umgebung. Der Typ der Umgebung hingegen hatte keinen Einfluss auf die Verhaltensdiversität, möglicherweise aufgrund der Futtersituation oder des variablen Sozialverhaltens. Ich konnte auch zeigen, dass

verschiedene Zebrafischstämme eine Präferenz für die strukturierte Umgebung haben. Interessanterweise zeigten Eilandbarben und Zebrafische Hin- und Herschwimmen, eine mögliche Stereotypie, nur in der Umgebung ohne Strukturen. Weiter unterschieden sich Eilandbarben und Zebrafische in der Nutzung der Wassersäule. Ich fand also sowohl bei den Füchsen als auch bei den Fischen eine Präferenz für eine strukturierte Umgebung, die das natürliche Habitat simuliert.

Die Dissertation hat gezeigt, dass das Konzept des Environmental Enrichment, das darauf basiert Umweltreize zu identifizieren, um artgerechte Haltungsbedingungen zu entwickeln und Verhaltensstörungen zu verhindern, bei Wirbeltierarten verschiedener Taxa angewendet werden kann. Die Resultate stützen die Hypothese, dass eine stimulierende Umgebung wichtig ist für die normale Hirnentwicklung und somit auch für die Entwicklung von normalem Verhalten.

GENEREAL INTRODUCTION

GENERAL INTRODUCTION

Species-adequate housing of zoo animals, pet animals and laboratory animals using environmental enrichment

The welfare of captive animals is an important issue of scientific research in animal behaviour. Animal welfare concerns are highly relevant regarding the millions of animals that are used by humans for reasons of food, research, entertainment and companionship. Keeping animals in captivity raises biological and ethical questions when inadequate housing compromises the animals' health and causes suffering (Mason, 2010). The behaviour of captive animals was shaped by the environmental conditions the animals' ancestors evolved in (Wechsler, 2007). The ability of animals to adapt to situations in captivity is constrained by these conditions, and when housing environments overtax the animal's adaptability physiological and immunological dysfunctions, chronic stress, behavioural disturbances and coordination disorders of the central nervous system can be the consequences (Stauffacher, 1998). Current forms of animal housing overtax many animals as behavioural problems are widely reported in zoo, laboratory, and pet animals (Casamitjana, 2004; Balcombe, 2006; Mason, 2010). Therefore, to enhance the welfare of captive animals, husbandry including enclosures, cages or aquaria have to be adjusted to the animals' behavioural and ecological needs.

Environmental enrichment

Environmental enrichment is a general philosophy of animal husbandry. The overall goal of environmental enrichment is to improve housing conditions by providing specific environmental stimuli, and research on environmental enrichment aims at identifying stimuli that allow species-specific behaviour (Shepherdson, 1998). It has been postulated that the lack of such stimuli or of critical resources that facilitate species-typical behaviour plays a role in the occurrence of behavioural disturbances (Mason, 1991; Casamitjana, 2004). Very often, environmental enrichment is applied with the purpose to reduce, to abolish or ideally prevent behavioural disturbances such as stereotypies (Mason et al., 2007). The specific goals of environmental enrichment are (a) to enhance behavioural

diversity, (b) to enhance the range and patterns of normal behaviour (i.e. behaviour that animals show under natural conditions), (c) to decrease abnormal behaviour patterns, (d) to increase the use of the environment, and (e) to increase the ability to better cope with challenges (Young, 2003).

The significance of "normal" behaviour

The fundamental aspect of the importance of "showing normal (or natural) behaviour" is intensively debated in the scientific literature, since it is controversial which natural behaviours are beneficial in captivity and which are not (Dawkins, 2006). In fact, some behavioural and genetic adaptation to humans and the captive environment have occurred during the process of domestication. Domestication was defined by Price (1984) "*as the process by which a population of animals becomes adapted to man and to the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events recurring during each generation.*" However, clear evidence that animals living since generations in captivity have retained their natural behavioural repertoire were provided by studies on farm animals. When they were given the opportunity to live in a semi-natural environment, they showed similar behaviour to their wild ancestors (e.g. in domestic pigs Stolba and Woodgush, 1989; in quails Schmid and Wechsler, 1997). Price (1999) suggests that behavioural differences between wild and domestic animals are rather quantitative in character and best explained by differences in response thresholds.

The different opinions, which have been raised concerning the significance of behavioural diversity and "normal" behaviour, can be summarized as follows. The Farm Animal Welfare Council (1993) lists the ability to perform most natural patterns of behaviour as one of the essential five freedoms (1. freedom from hunger and thirst, 2. freedom from discomfort, 3. freedom from pain, injury or disease, 4. freedom to express normal behaviour, and 5. freedom from fear and distress). Kiley-Worthington (1989) agues that in captivity animals should be able to show the whole range of their behavioural repertoire for their wellbeing. Newberry (1995) suggests a more functional approach that focuses on the plasticity of an animal's behaviour to adapt to captive environments. Dawkins (2006) advocates an evolutionary approach and a fruitful input from behavioural ecologists to understand an animal's natural behaviour and strategies. Mason et al. (2007) emphasize that natural behaviour patterns animals show in the wild have to be taken into account when using environmental enrichment to tackle stereotypic behaviour. Wechsler (2007) pointed out that knowledge on animal behaviour and

motivation is crucial to use normal behaviour as an indicator to measure animal welfare. Nevertheless, although animals that are constrained to perform natural behaviour are supposed to suffer, it is not mandatory that enhanced welfare is causally determined by the opportunity to exhibit all behaviours of the wild animals repertoire (Veasey et al., 1996). In captivity not all behaviours seem to be equally important for good welfare, e.g. anti-predator behaviour or infanticide, therefore criteria have to be formulated which behaviours enhance welfare (Dawkins, 1998). Still, for animals bred in zoos for reintroduction it is crucial to maintain the whole range of behavioural diversity, including predator and anti-predator behaviour (Rabin, 2003).

Research to uncover causes and mechanisms involved in the development of behavioural disturbances such as stereotypies is a highly complex issue (Würbel, 2006), and satisfying solutions to abolish stereotypies are difficult to find. There is also evidence that environmental complexity has a positive effect on shaping behaviour and brain development during early development (Lewis, 2006). Therefore, allowing the animals to perform most of their natural behaviours, predominantly during the periods of early development, may be very important and the best way to prevent the development of behavioural disturbances and thus to reach good welfare.

Enrichment types and research areas

To provide the animals with a stimulating environment, different types of enrichment can be applied: feeding enrichment, such as providing foraging and feeding opportunities, structural enrichment to increase structural complexity, social enrichment adapted to the species social organisation, occupational enrichment, such as exercises, and sensory enrichment, such as olfactory stimulation (Young, 2003). Combining different types of enrichment and varying enrichment elements can generate variability as well as complexity, and may prevent habituation to enrichment elements (Maple and Perkins, 1996).

The concept of environmental enrichment has been studied predominantly in zoo, farm and laboratory animals. A gap analysis by Schetini de Azevedo et al. (2007), intended to detect biases in research focus, revealed that approx. 90 % of all studies on environmental enrichment were conducted with mammals, the remaining 10 % with birds, fish, reptiles, and invertebrates. Since many common laboratory, and most farm species are mammals, it is not surprising that the majority of studies

focussed on this taxonomic group. However, probably all animals kept in captivity would benefit from research on environmental enrichment. This may be especially noteworthy for fish that are kept by humans in their millions (Conte, 2004; Huntingford et al., 2006).

The motivation for research on environmental enrichment and its application varies between the different areas of animal husbandry. Whereas laboratory animal research focuses on validity and applicability of research results, in farm animals productivity and public image are the main purpose. In zoos emphasis is placed on housing conditions that should enable animals to engage in natural behaviour and provide visitors with insights into the animals' natural habitat (Newberry, 1995). In the following, I will focus on zoo animals with emphasis on carnivores, and on laboratory and pet animals with emphasis on fish in which environmental enrichment is still poorly studied.

Environmental enrichment in zoo animals

The frequent occurrence of stereotypic behaviours or other abnormal repetitive behaviours (ARBs) in zoo animals (Mason and Latham, 2004; Mason et al., 2007) and increasing ethical concerns advocated the development of environmental enrichment (Hediger, 1950; Shepherdson, 1998; Swaisgood and Shepherdson, 2005). A number of literature reviews show that environmental enrichment has been widely studied and successfully applied in zoo animals (Chamove, 1989; Carlstead and Shepherdson, 1994; Newberry, 1995; Swaisgood and Shepherdson, 2005; Shyne, 2006; Mason et al., 2007; Schetini de Azevedo et al., 2007). For zoo animals, a naturalistic approach to promote natural behaviour has been proposed by Hutchins et al. (1984). This approach aims at establishing natural-looking enclosures where the complexity and unpredictability of the species' natural habitat is imitated, and structuring and furnishing is adjusted to the animals' behavioural and ecological needs. Today, a main issue of modern zoos is to provide people with information about the biology of their animals and their natural habitats. Therefore, animals are more and more housed in enclosures portraying their natural environment (Robinson 1998). Still, in addition to such an educative rationale, simulating natural structures or feeding resources using artificial structures provide important opportunities for animals to engage in natural behaviour, and moreover may give the animals some control over their environment (Markowitz and Aday, 1998). A combination of the naturalistic approach and a more technical approach using automatic or mechanical devices and artificial structures (e.g.

climbing structures) seems to be most promising in enhancing variability and complexity in enclosures (Maple and Perkins, 1996; Shepherdson, 1998).

Structural and feeding enrichment

Among the different enrichment types, zoos apply most frequently structural and feeding enrichment (Schetini de Azevedo et al., 2007). By using structural enrichment, the physical complexity of the environment can be increased and biologically relevant information can be added to an animal's enclosure such that opportunities for exploration increase (Swaisgood, 2006). Structures can fulfil a wide range of functions, they make space vertically and horizontally accessible for animals; they divide the enclosure into functionally different subareas; they serve as barriers or hiding places from conspecifics, the public, and keepers; they provide shade or shelter, climbing opportunities or lookouts (Maple and Perkins, 1996; Swaisgood and Shepherdson, 2005). Hediger (1950) pointed out that rather quality of space than quantity of space is important. However, requirements for enclosure size and responses to restricted space may vary between species. In primates, for example, enhancing complexity of space may compensate for restricted space to some degree as they possess well developed manipulative and cognitive skills (Hosey, 2005). In a survey of gorillas and orangutans in forty-one zoos it turned out that activity was influenced by stationary or moveable and temporary objects rather than by the size or construction of the enclosure (Wilson, 1982). Nevertheless, spacious enclosures offer more possibilities to add various structures with biologically relevant information, and enable animals to withdraw from threatening situations or disturbances (Carlstead, 1991). Frézard and Le Pape (2003) observed in wolves a more natural time budget in bigger enclosures because they were less disturbed by visitors or keepers. Carnivores with a naturally wide-ranging lifestyle seem to be especially susceptible to behavioural problems in captivity, and therefore providing more space would be one means to improve welfare of such species (Clubb and Mason, 2007).

Although environmental enrichment often reduces and not eliminates abnormal behaviour (Swaisgood, 2006), providing animals with feeding enrichments seems to be an effective way to improve conditions of a wide range of animals in captivity (Lindburg, 1998). Under natural conditions, foraging accounts for a high percentage of the activity budget, in other words, food acquisition is usually a time consuming and challenging task (Herbers, 1981; Shepherdson et al., 1993; Lindburg, 1998). In captivity, on the other hand, animals do not have to make a great effort for searching,

acquisition and processing of food. Moreover, food is mostly provided in a highly predictable way and on a fixed time schedule; thus various natural foraging behaviours and behavioural patterns cannot be exhibited (Carlstead, 1996).

Abnormal behaviours such as stereotypies are often explained to be a consequence of frustrated appetitive and feeding behaviours caused by inadequate feeding regimes (Swaisgood, 2006). For example, licking of non-food objects is a frequently observed abnormal behaviour in captive exotic ungulates (Bashaw et al., 2001). Providing Masai giraffes (*Giraffa camelopardalis tippelskirchi*) with feeding resources that demanded more oral manipulation to obtain feed resulted in a reduced frequency of the undesired abnormal behaviour (Fernandez et al., 2008). In birds, oral and locomotor stereotypies are often observed under restricted conditions, with oral stereotypies apparently being related to limited foraging opportunities, and locomotor stereotypies to the lack of space and physical complexity (Keiper, 1969 cited in Meehan et al., 2004). When young orange-winged Amazon parrots (*Amazona amazonica*) were offered a combination of feeding and structural enrichment, facilitating foraging and locomotor behaviour, they developed significantly less stereotypic behaviour than individuals that did not get such enrichment (Meehan et al., 2004).

To elicit appetitive components of foraging behaviour or manipulative behaviour different forms of feeders have been employed in various species, predominantly in primates and carnivores. For example, probe feeders and different forms of puzzle feeders have been employed to challenge manipulative and learning skills in several primate species (for a review see Reinhardt and Roberts, 1997). In chimpanzees a tool task successfully elicited behaviours similar to termite fishing in the wild, and moreover involved cognitive stimulation (Celli et al., 2003). A further advantage of feeders is the possibility to provide food independent of human presence and at varying times during the day, thus diminishing predictability of feeding and unreliable signals relating to feeding (Bassett and Buchanan-Smith, 2007).

In carnivores, pacing is the most prevalent abnormal repetitive behaviour and is suggested to be related to frustrated appetitive behaviour, however, other alternative motivational explanations are discussed such as frustrated escape or ranging behaviour (Clubb, 2005; Clubb and Mason, 2007). Many carnivores are considered to be very curious and exploratory animals (Glickman and Sroges,

1966). Feeding enrichment thus has been repeatedly tested in such species (Schetini de Azevedo et al., 2007). Feeding enrichment proved to influence the behaviour of captive carnivores in various ways such as enhancing use of space (Mellen et al., 1981; Lindburg, 1988), reducing stereotypies (Forthman et al., 1992; Shepherdson et al., 1993; Blount and Taylor, 2000; Jenny and Schmid, 2002; McPhee, 2002; Swaisgood et al., 2005), eliciting hunting or appetitive behaviour (Markowitz and LaForse, 1987; Powell, 1995; Williams et al., 1996; Hartmann-Furter, 2000; Bashaw et al., 2003; Cummings et al., 2007), or enhancing the amount of time animals engage in food searching (Carlstead, 1991; Langenhorst, 1997; Fischbacher and Schmid, 1999). Nevertheless, some studies also reported that feeding enrichment did not generate the desired results such as reducing stereotypic behaviour or eliciting foraging behaviour (e.g. Carlstead et al., 1991; Bashaw et al., 2003; Burgener et al., 2008). Therefore, it is important to take into account the natural foraging behaviour and strategies of an animal, i.e. how, when, where and how often it searches for food, and when applying feeding enrichments. For example, many bears posses strong manipulative skills to find food and are motivated to explore feeding places. Therefore, devices that can be manipulated are more effective in eliciting food searching behaviour than automatic feeders that just release food (Carlstead et al., 1991). To provide captive carnivores with the opportunity to show the full range of hunting behaviour (travelling, localising, catching, killing and eating) is difficult. In wildcats (*Felis s. silvestris*) a wide range of natural hunting behaviours could be elicited with the aid of electronic feeders, which simulated the feeding situation in the wild, and moreover, the development of abnormal behaviours could be prevented (Hartmann-Furter, 2000). Enhancing variation in feeding enrichments might be an important means to prevent habituation to feeding regimes (Carlstead et al., 1991; Powell, 1995). As behavioural problems of captive carnivores might have different causes, different types of enrichment should be tested and eventually be combined (Clubb and Mason, 2007).

Environmental enrichment in pet and laboratory animals

Increasing concerns for laboratory animal welfare and evidence that current standard housing conditions may impair the validity of scientific results have influenced the discussion about housing standards and guidelines of laboratory animals (Sherwin, 2004; Balcombe, 2006; Richter et al., 2009). Standard housing systems for laboratory animals are rather designed on the basis of economic and practical aspects than on biological aspects. There is evidence that impoverished environments lead to an impairment of normal brain development, and therefore leading to altered brain functions and

behaviour (Würbel, 2001). In rodents, it was suggested that barren standard housing conditions overtax their capacity to adapt to the environment, and that as a consequence they develop abnormal behaviours such as stereotypies that are an indicator of impaired welfare (Würbel et al., 1998; Callard et al., 2000; Garner and Mason, 2002; Waiblinger and König, 2004). The various stereotypies observed in many rodents are hypothesised to be escape attempts from the barren cages in search of shelter or conspecifics (Würbel, 2006). Evidence for this is given by a study on mice by Lewis and Hurst (2004) where they showed that mice preferred to chew on external bars that opened daily to those that did not open. Olsson et al. (2003) proposed an ethological approach to understand the behavioural biology of laboratory animals and to investigate which features of the environment are important in laboratory husbandry to improve housing systems or even establish new systems.

The type of enrichment tested in laboratory animals has predominantly been structural enrichment (Schetini de Azevedo et al., 2007). Most information about environmental enrichment in laboratory animals originates from rodents (rats and mice) because these account for 90% of all vertebrate animals used for laboratory research (Balcombe, 2006). In laboratory mice and rats different structural enrichments such as nests, nesting material, shelter, climbing structures, elevated platforms, opportunities to explore, and social enrichment were tested. Such enrichment proved to be effective in reducing stereotypies, fostering normal brain development, reducing fear of novelty, anxiety or stress, and enhancing learning and memory (reviewed in Olsson and Dahlborn, 2002; Balcombe, 2006). Size and shape of cages affect species differently. Whereas in bank voles (*Clethrionomys glareolus*) structural complexity and not cage size influenced the level of stereotypies observed (Ödberg, 1987), in European starlings (*Sturnus vulgaris*) larger and long cages were associated with fewer stereotypies (Asher et al., 2009). Although rodents are at the focus of laboratory welfare research, other laboratory animals have been found to benefit from environmental enrichment (Reinhardt, 2002; in rabbits, reviewed in Verga et al., 2007; in passerine birds, reviewed in Bateson and Feenders, 2010); evidence that is also useful in research of pet animals.

For quite some time welfare concerns for pet animals have also increased due to their growing number, although pressure from the public is less strong and control is more difficult than in farm, zoo or laboratory animals (Steiger, 2008). Pet animals mostly live in a highly artificial environment dominated by humans and have adapted to some extent to man-made housing conditions (Newberry,

1995; Sachser, 1998). However, the process of domestication differs greatly between the various species kept as pet animals. Whereas for example guinea pigs have been domesticated since hundreds of generations, parrots are still regarded as wild animals, what could make their housing even more demanding (Sachser, 1998; Engebretson, 2006). Broom (2010) furthermore suggests that the different cognitive abilities of animals kept in captivity should be taken into account when housing issues are investigated. The impressive cognitive abilities of African grey parrots (*Psittacus erithacus*) are described by Pepperberg (2002), but there is also increasing evidence for cognitive skills in fish (Broglio et al., 2003; Broom, 2010). Main problems with pets reported by pet owners are housing, health and behavioural problems (Steiger, 2008). Comparable to farm animals, also pet animals have retained their behavioural needs and their welfare can be improved by appropriate environmental enrichment. For example, domestic cats should be enabled to use the vertical dimension indoors, as they climb well and use elevated places as vantage points (Rochlitz, 2005). Many pet animals are social animals originally living in groups; however, they are often kept alone what causes behavioural and health problems (e.g. for a review on pet birds see van Hoek, 1998; for a survey on pet rabbits see Schepers et al., 2009). Whereas pet mammals and pet birds were given some attention regarding welfare recently, ornamental fish are rather neglected, although they are kept in their millions in private aquaria (Livengood and Chapman, 2007).

Fish are not only popular pet animals, but they are also increasingly used as animal models in research (Livengood and Chapman, 2007; Williams et al., 2009). In pet fish the following problems occur: inappropriate densities or species combinations, poor water quality, inappropriate food (amount and food regime), disease, and inadequate furnishing of aquaria. Laboratory fish experience similarly barren housing conditions as rodents under traditional lab conditions (Huntingford et al., 2006; Spence et al., 2008). Considering the complex behaviour, physiology and brain anatomy of fish, it can be assumed that fish also suffer from inadequate housing conditions (Huntingford et al., 2006; Broom, 2007). Research on environmental enrichment in fish is only at the beginning, and in contrast to many mammal species fundamental knowledge of the biology of fish species kept in captivity is still limited (Williams et al., 2009). Some evidence that fish benefit from environmental enrichment can be derived from studies in aquaculture where structural enrichment reduced aggressiveness or stress, and a combination of feeding and environmental enrichment during rearing enhanced the survival rate of fish that were released in the wild (Ashley, 2007). Spatial cues and a variable feeding regime generated

fish with more flexible feeding behaviour, with a better recovery from a stress situation and a more flexible space use (Braithwaite and Salvanes, 2005). To reduce aggression during feeding sessions, automatic feeders are proposed that dispend food rations repeatedly throughout the day (Greaves and Tuene, 2001). There is also evidence that environmental enrichment influences brain development in fish. Enriched juvenile rainbow trout (*Oncorhynchus mykiss*) grew brains with significantly larger cerebella and showed different locomotor behaviours compared to individuals reared in conventional tanks (Kihslinger and Nevitt, 2006). However, the question remains which environmental enrichment is beneficial to which fish species. For instance, in a study with stickleback (*Gasterosteus aculeatus*) and the European minnow (*Phoxinus phoxinus*), the latter ate more and grew faster in the presence of cover than in its absence. In sticklebacks no such effect occurred. These results probably reflected the different lifestyles of the two species (Wootton et al., 2006). Therefore, inter-specific differences regarding behavioural and ecological needs have to be taken into account when environmental enrichment is tested in fish.

Thesis outline

To assess the significance of environmental enrichment in promoting and maintaining species-typical behaviour and behavioural diversity in captive animals, it is feasible to adopt a naturalistic approach, i.e. to investigate animals under naturalistic conditions and providing them with stimulating environments (or naturalistic stimuli) that are based on the natural habitat and the natural behaviour of the studied species. It is essential to systematically test specific naturalistic stimuli for their potential to elicit species-typical behaviour. In addition, it is helpful to use a comparative approach, i.e. investigating different species of different taxa to verify the general principle of using naturalistic stimuli to promote species-typical behaviour, and furthermore investigating the issue in different areas such as zoo, laboratory and pet animal housing. To verify the concept on environmental enrichment, I conducted a series of experiments using (1) a naturalistic approach allowing natural behaviours, and enhanced behavioural diversity and enclosure use, and (2) a comparative approach using different species of different taxa. In all my experiments I focussed on behavioural measures, and I used normal (or natural) behaviour and the absence of abnormal behaviour as a proxy of good welfare.

The concept of environmental enrichment has been used in a wide variety of zoo, farm and laboratory animals. However, there is a considerable bias towards specific species that get enriched (Schetini de

Azevedo et al., 2007). In zoos, environmental enrichment has been examined mainly in primates, parrots and carnivores such as ursidae and felids. Within carnivores small candis such as the red fox (*Vulpes vulpes*) are hardly investigated although foxes are a common species in wildlife parks (pers. observation). In laboratory and pet animals, studies on environmental enrichment have been conducted predominantly in mammals such as rodents. In contrast, information about environmental enrichment in fish is scarce, although they are widely used by humans for reasons of research and pleasure.

The wildlife park Langenberg was planning a big new naturalistic enclosure for red foxes. This offered me the possibility to observe a group of red foxes in a smaller test enclosure, which simulated a naturalistic habitat of red foxes and was situated outside the official park. In the test enclosure, I was able to first experimentally examine the influence of a feeding enrichment and two structural enrichments on behaviour, behavioural diversity, and enclosure use of foxes, and then verify the influence of these enrichments on their behaviour in the new enclosure, which was built based on the result obtained in the test enclosure.

Fish offer the great possibility to simultaneously investigate different species because it needs relatively little facility space to house them. Therefore, I focussed in further experiments on different species of the genus *Barbus* and on zebrafish (*Danio rerio*), both common pet fish; the latter is also a widely used animal model in laboratory research. In different aquaria provided with naturalistic equipment, I examined the preferences for structural enrichment.

In carnivore mammals the variety of ecological and behavioural needs is respectable, in fish the diversity is enormous. However, a common attribute of carnivore mammals and many pet fish species is their highly explorative nature. Both, red foxes and barbs are "small opportunistic omnivores". Red foxes are less specialised than felids but rather similar to bears showing a wide trophic niche. Although they may live in social groups, predominantly in urban areas, such as wolves, they are solitary foragers. Barbs usually form groups and forage also on a wide range of prey items using different levels of the water column. Generally, smaller species are heavily suffering from predators, including humans. By using and comparing different species such as foxes and fish as model species

for experimentation, it might be possible to give general evidence about the influence of feeding and structural enrichments on behaviour in opportunistic and highly explorative animals.

Red foxes are opportunistic carnivores and use a variety of foraging strategies; they hunt small mammals or birds, but also feed on windfall or compost heaps (Contesse et al., 2004). Under natural conditions, food resources are mostly unpredictable in time and space and finding food is time consuming for foxes. In captivity, feeding enrichments using artificial devices can add variability and complexity to the animal's environment and elicit species-specific behaviour. **Chapter 1** deals with a varied feeding enrichment that is based on the natural foraging strategies of foxes and on the temporal and spatial unpredictability of food. Enrichment consisted of an electronic feeder that provided food alone or in combination with a self-service food box, with scattered and hidden food, or with an electronic dispenser. I compared the four feeding enrichments with a conventional feeding regarding their effects on foraging and feeding activity and on behavioural diversity. I was able to show that in opportunistic carnivores natural foraging and feeding behaviour can be stimulated by simple feeding enrichment strategies, and that foraging behaviour is stimulated most when food delivery is unpredictable both in space and time.

Red foxes are regarded as a highly adaptable species that also thrives in urban areas. Nevertheless, by adopting a mostly nocturnal lifestyle red foxes avoid human activities and they prefer highly structured, cover-rich areas, and secure hiding and rearing places (Gloor, 2002). However, this behaviour might contrast with the desire of the public to observe the animals; a conflict that might be partly solved when the animals are fed during the day. Therefore, structural enrichment fulfils different important functions such as making space accessible or providing cover and shelter, and thus might help to prevent additional stress when food is provided during the day. Presenting the animals with environments that vary systematically helps to identify the conditions that meet the animals' behavioural and ecological needs. **Chapter 2** is divided into two parts. In the first part, I examined two types of structural enrichment that simulate structures occurring in the natural habitat of foxes, allowing the foxes to range safely in their territory. The first experiment dealt with structures that simulate long structures such as hedges or walls. The second experiment dealt with structures that simulate cover such as bushes or thickets. I used behavioural measures and the use of space to evaluate the consequences of environmental modifications. The foxes showed a preference for

moving along long wooden structures and shifted their use of sectors depending on the location of cover structures. Offering structures imitating naturalistic stimuli thus had a profound effect upon the foxes' use of the enclosure. In the second part of this chapter, I verified the results of the first part in a newly built outdoor enclosure into which the foxes were transferred. I examined the preference for different structures and the trend in activity during the initial phase after transfer to the bigger enclosure, representing a new and potentially challenging situation. The foxes showed a significant overall preference for structures and a preference for sectors containing structural enrichment compared to corresponding areas with no structural enrichment. In the course of the observation period, the foxes showed increased use of open space and increased activity.

Zebrafish and barbs are both common pet fish. Moreover, zebrafish are increasingly used in research as animal models. Genetics and neurobiology of zebrafish are well-studied, and information about their natural biology and laboratory husbandry is available (Lawrence, 2007; Spence et al., 2008). However, there is little scientific information about the welfare of fish in standard laboratory housing. In rodents, standard barren cages often cause stereotypies, what raised concern that standard housing and husbandry may reduce the validity of test results (Würbel, 2001; Sherwin, 2004). As zebrafish are widely used in neurobiology and developmental biology (Vascotto et al., 1997), this issue should be urgently considered also in fish housing. Information on husbandry of ornamental fish in general and barbs in particular is available predominantly from aquarist literature (Kortmulder, 1981; Riehl and Baensch, 1983). It is therefore essential to conduct scientific experiments on species-adequate furnishing of aquaria. Preference tests are a well-established tool to investigate conditions that might improve the animal's welfare. To do so, animals are provided with one or more situations or resources between which they can choose. It is assumed that they show what they want through their behaviour (Dawkins, 2004). Both zebrafish and barbs occur in well-vegetated shallow water bodies in the wild. In **chapter 3**, I investigated the preference for enriched environments in a choice experiment. I offered the fish the possibility to choose between a barren compartment and a structured compartment. I used six different barbs species and zebrafish. I expected that all barb species and zebrafish show a preference for the structured compartment. Furthermore I expected a higher behavioural diversity and a different use of space in the structured compartment. The results showed that all groups of barb species, except one, and zebrafish showed a preference for the structured compartment. Nevertheless, over all species, behavioural diversity and space use did not differ significantly between

the empty and the structured compartment. Despite the fact that the species studied belong to the same family and occur in similar habitats, no general behavioural pattern emerged. One group even avoided the structured compartment, and also substantial differences in space use occurred. In particular, I observed striking behavioural differences in social behaviour. Therefore, it was indicated to examine the fish on a species rather than on a genus level to reach general conclusions about the behavioural and ecological needs of a specific fish species. Moreover, several different strains of zebrafish exist for scientific research. Here, the question remains if different strains show differences in preference, behaviour and space use. Therefore, I concentrated on two species for further experiments: checker barbs (*Barbus oligolepis*) as a common pet species, and zebrafish including four different strains as a lab and pet species. The results of these experiments are presented in **chapter 4.** I used the same design for the choice experiment as in the precedent chapter. I was able to confirm that both all strains of zebrafish and checker barbs show a significant preference for a complex (naturalistic) environment. 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. The results suggest that environmental enrichment should be tested on a species level in fish.

REFERENCES

Asher, L., Davies, G.T.O., Bertenshaw, C.E., Cox, M.A.A., Bateson, M., 2009. The effects of cage volume and cage shape on the condition and behaviour of captive European starlings (Sturnus vulgaris). Appl. Anim. Behav. Sci. 116, 286-294.

Ashley, P.J., 2007. Fish welfare: Current issues in aquaculture. Appl. Anim. Behav. Sci. 104, 199-235.

Balcombe, J.P., 2006. Laboratory environments and rodents' behavioural needs: a review. Lab. Anim. 40, 217-235.

Bashaw, M.J., Bloomsmith, M.A., Marr, M.J., Maple, T.L., 2003. Feeding enrichment experiment with captive large felids. Zoo Biol. 22, 189–198.

Bashaw, M.J., Tarou, L.R., Maki, T.S., Maple, T.L., 2001. A survey assessment of variables related to stereotypy in captive giraffe and okapi. Appl. Anim. Behav. Sci. 73, 235-247.

Bassett, L., Buchanan-Smith, H.M., 2007. Effects of predictability on the welfare of captive animals. Appl. Anim. Behav. Sci. 102, 223-245.

Bateson, M., Feenders, G., 2010. The Use of Passerine Bird Species in Laboratory Research: Implications of Basic Biology for Husbandry and Welfare. Ilar J. 51, 394-408.

Blount, J.D., Taylor, N.J., 2000. The relative effectiveness of manipulable feeders and olfactory enrichment for Kinkajous (*Potos flavus*): a preliminary study at Newquay Zoo. Int. Zoo. Yearb. 37, 381-394.

Braithwaite, V.A., Salvanes, A.G.V., 2005. Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. Proc. R. Soc. B-Biol. Sci. 272, 1107-1113.

Broglio, C., Rodriguez, F., Salas, C., 2003. Spatial cognition and its neural basis in teleost fishes. Fish Fish. 4, 247-255.

Broom, D.M., 2007. Cognitive ability and sentience: Which aquatic animals should be protected? Dis. Aqaut. Organ. 75, 99-108.

Broom, D.M., 2010. Cognitive ability and awareness in domestic animals and decisions about obligations to animals. Appl. Anim. Behav. Sci. 126, 1-11.

Burgener, N., Gusset, M., Schmid, H., 2008. Frustrated appetitive foraging behavior, stereotypic pacing, and fecal glucocorticoid levels in snow leopards (Uncia uncia) in the Zurich zoo. J. Appl. Anim. Welf. Sci. 11, 74-83.

Callard, M.D., Bursten, S.N., Price, E.O., 2000. Repetitive backflipping behaviour in captive roof rats (Rattus rattus) and the effects of cage enrichment. Anim. Welfare 9, 139-152.

Carlstead, K., 1991. Husbandry of the Fennec fox *Fennecus zerda*: Environmental conditions influencing stereotypic behaviour. Int. Zoo. Yearb. 30, 202-207.

Carlstead, K., 1996. Effects of Captivity on the Behavior of Wild Mammals, In: Kleinmann, D.G., Allen, M.E., Thompson, K.V., Lumpkin, S. (Ed.), Wild mammals in captivity: principles and techniques, University of Chicago Press, Chicago and London.

Carlstead, K., Seidensticker, J., Baldwin, R., 1991. Environmental Enrichment for Zoo Bears. Zoo Biol. 10, 103-116.

Carlstead, K., Shepherdson, D., 1994. Effects of Environmental Enrichment on Reproduction. Zoo Biol. 13, 447-458.

Casamitjana, J., 2004. Aquatic Zoos. A Critical Study of UK Public Aquaria in the Year 2004. The Captive Animals' Protection Society.

Celli, M.L., Tomonaga, M., Udono, T., Teramoto, M., Nagano, K., 2003. Tool use task as environmental enrichment for captive chimpanzees. Appl. Anim. Behav. Sci. 81, 171-182.

Chamove, A.S., 1989. Environmental enrichment: A review. Anim. Tech. 40, 155-178.

Clubb, R., Mason, G.J., 2007. Natural behavioural biology as a risk factor in carnivore welfare: How analysing species differences could help zoos improve enclosures. Appl. Anim. Behav. Sci. 102, 303-328.

Clubb, R., Vickery, S.S., 2005. Locomotory stereotypies in carnivores: does pacing stem from hunting, ranging or frustrated escape?, In: Mason, G., Rushen, J. (Ed.), Stereotypic Behaviour in Captive Animals: Fundamentals and Applications for Welfare, 2nd edn., CAB International, Wallingford.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86, 205-223.

Contesse, P., Hegglin, D., Gloor, S., Bontadina, F., Deplazes, P., 2004. The diet of urban foxes (*Vulpes vulpes*) and the availabilty of anthropogenic food in the city of Zurich, Switzerland. Mamm. Biol. 69, 81-95.

Cummings, D., Brown, J.L., Rodden, M.D., Songasen, N., 2007. Behavioral and physiologic responses to environmental enrichment in the maned wolf (*Chrysocyon brachyurus*). Zoo Biol. 26, 331-343.

Dawkins, M.S., 1998. Evolution and Animal Welfare. Q. Rev. Biol. 73, 305-328.

Dawkins, M.S., 2004. Using behaviour to asses animal welfare. Anim. Welfare 13, S3-7.

Dawkins, M.S., 2006. A user's guide to animal welfare science. Trends Ecol. Evol. 21, 77-28.

Engebretson, M., 2006. The welfare and suitability of parrots as companion animals: a review. Anim. Welfare 15, 263-276.

Farm Animal Welfare Council, 1993. Second report on priorities for research and development in farm animal welfare. FAWC, Ministry of Agriculture, Fisheries and Food, Tolworth (Now DEFRA, London).

Fernandez, L.T., Bashaw, M.J., Sartor, R.L., Bouwens, N.R., Maki, T.S., 2008. Tongue twisters: Feeding enrichment to reduce oral stereotypy in giraffe. Zoo Biol. 27, 200-212.

Fischbacher, M., Schmid, H., 1999. Feeding Enrichment and Stereotypic Behavior in Spectacled Bears. Zoo Biol. 18, 363–371.

Forthman, D.L., Elder, S.D., Bakeman, R., Kurkowski, T.W., Noble, C.C., Winslow, S.W., 1992. Effects of feeding enrichment on behavior of three species of captive bears. Zoo Biol. 11, 187-195.

Frézard, A., Le Pape, G., 2003. Contribution to the Welfare of Captive Wolves (Canis lupus lupus): A Behavioral Comparison of Six Wolf Packs. Zoo Biol. 22, 33–44.

Garner, J.P., Mason, G.J., 2002. Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. Behav. Brain Res. 136, 83-92.

Glickman, S.E., Sroges, R.W., 1966. Curiosity in zoo animals. Behaviour 26, 151-&.

Gloor, S., 2002. The rise of urban foxes (*Vulpes vulpes*) in Switzerland and ecological and parasitological aspects of a fox population in the recently colonised city of Zurich, University of Zurich, Switzerland, Zurich.

Greaves, K., Tuene, S., 2001. The form and context of aggressive behaviour in farmed Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture 193, 139-147.

Hartmann-Furter, M., 2000. A species-specific feeding technique for European wildcats (*Felis s. sylvestris*) in captivity. Säugetierkd. Inf. 4, 567-575.

Hediger, H., 1950. Wild animals in captivity. Butterworths, London.

Herbers, J.M., 1981. Time resources and laziness in animals. Oecologia 49, 252-262.

Hosey, G.R., 2005. How does the zoo environment affect the behaviour of captive primates? Appl. Anim. Behav. Sci. 90, 107-129.

Huntingford, F.A., Adams, C., Braithewaite, V.A., Kadri, S., Pottinger, G., Sandoe, P., Turnbull, J.F., 2006. Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Hutchins, M., Hancocks, D., Crockett, C., 1984. Naturalistic solutions to the behavioral problems of captive animals. Zool. Garten N.F. 54, 28-42.

Jenny, S., Schmid, H., 2002. Effect of feeding boxes on the behavior of stereotyping amur tigers (*Panthera tigris altaica*) in the Zurich Zoo, Zurich, Switzerland. Zoo Biol. 21, 573–584.

Kihslinger, R.L., Nevitt, G.A., 2006. Early rearing environment impacts cerebellar growth in juvenile salmon. J. Exp. Biol. 209, 504-509.

Kiley-Worthington, M., 1989. Ecological, Ethological, and Ethically Sound Environments for Animals: Toward Symbiosis. J. Agr. Ethic. 2, 323-347.

Kortmulder, K., 1981. Etho-Ecology of 17 Barbus Species (Pisces, Cyprinidae). Neth. J. Zool. 32, 144-168.

Langenhorst, T., 1997. Effects of a behavioural enrichment program on the stereotypic behaviour of brown bears (*Ursus arctos*). Zool. Garten N.F. 67, 341-354.

Lawrence, C., 2007. The husbandry of zebrafish (Danio rerio): A review. Aquaculture 269, 1-20.

Lewis, M.H., Presti, M.F., Lewis, J.B., Turner, C.A. , 2006. The Neurobiology of Stereotypy I: Environmental Complexity, In: Mason, G., Rushen, J. (Ed.), Stereotypic Behaviour in Captive Animals: Fundamentals and Applications for Welfare, 2nd ed.

Lewis, R.S., Hurst, J.L., 2004. The assessment of bar chewing as an escape behaviour in laboratory mice. Anim. Welfare 13, 19-25.

Lindburg, D.G., 1988. Improving the feeding of captive felines through application of field data. Zoo Biol. 7, 211-218.

Lindburg, D.G., 1998. Enrichment of captive mammals through provisioning, In: Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), Second nature: Environmental Enrichment for Captive Animals, Smithsonian Institution Press, Washington, pp. 262-276.

Livengood, E.J., Chapman, A., 2007. The Ornamental Fish Trade: An Introduction with Perspectives for Responsible Aquarium Fish Ownership. Department of Fisheries and Aquatic Sciences, Florida. http://edis.ifas.ufl.edu.

Maple, T., Perkins, L., 1996. Enclosure furnishing and structural environmental enrichment, In: Kleinmann, D., Allen, E., Thompson, K., Lumpkin, S. (Eds.), Wild mammals in captivity: principles and techniques, University of Chicago Press, Chigago (IL), USA, pp. 212-222.

Markowitz, H., Aday, C., 1998. Power for captive animals, In: Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), Second nature: Environmental Enrichment for Captive Animals, Smithsonian Institution Press.

Markowitz, H., LaForse, S., 1987. Artificial prey as behavioral enrichment devices for felines. Appl. Anim. Behav. Sci. 18, 31-43.

Mason, G., Clubb, R., Latham, N., Vickery, S., 2007. Why and how should we use environmental enrichment to tackle stereotypic behaviour? Appl. Anim. Behav. Sci. 102, 163-188.

Mason, G.J., 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015-1037.

Mason, G.J., 2010. Species differences in responses to captivity: stress, welfare and the comparative method. Trends Ecol. Evol. 25, 713-721.

Mason, G.J., Latham, N.R., 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim. Welfare 13, S57-S69.

McPhee, M.E., 2002. Intact carcasses as enrichment for large felids: effects on on- and off-exhibit behaviors. Zoo Biol. 21, 37-47.

Meehan, C.L., Garner, J.P., Mench, J.A., 2004. Environmental enrichment and development of cage stereotypy in Orange-winged Amazon parrots (Amazona amazonica). Dev. Psychobiol. 44, 209-218.

Mellen, J., Stevens, V., Markowitz, H., 1981. Environmental enrichments for servals, Indian elephants and Canadian otters. Int. Zoo. Yearb. 21, 196-201.

Newberry, R.C., 1995. Environmental enrichment: Increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44.

Ödberg, F.O., 1987. The Influence of cage size and environmental enrichment on the development of stereotypies in bank voles (*Clethrionomys glareolus*). Behav. Processes 14, 155-173.

Olsson, I.A.S., Dahlborn, K., 2002. Improving housing conditions for laboratory mice: a review of 'environmental enrichment. Lab. Anim. 36, 243-270.

Olsson, I.A.S., Nevison, C.M., Patterson-Kane, E.G., Sherwin, C.M., Van de Weerd, H.A., Wurbel, H., 2003. Understanding behaviour: the relevance of ethological approaches in laboratory animal science. Appl. Anim. Behav. Sci. 81, 245-264.

Pepperberg, I.M., 2002. Cognitive and communicative abilities of grey parrots. Curr. Dir. Psychol. 11, 83-87.

Powell, D.M., 1995. Preliminary evaluation of environmental enrichment techniques for african lions (Panthera leo). Anim. Welfare 4.

Price, E.O., 1984. Behavioral aspects of animal domestication. Q. Rev. Biol. 59, 1-32.

Price, E.O., 1999. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65, 245-271.

Rabin, L.A., 2003. Maintainig behavioural diversity in captivity for conservation natural behaviour management. Anim. Welfare 12.

Reinhardt, V., Reinhardt, A. (Ed.), 2002. Comfortable quarters for laboratory animals. Animal Welfare Institute, Washington SC.

Reinhardt, V., Roberts, A., 1997. Effective feeding enrichment for non-human primates: a brief review. Anim. Welfare 6, 265-272.

Richter, S.H., Garner, J.P., Wurbel, H., 2009. Environmental standardization: cure or cause of poor reproducibility in animal experiments? Nat. Methods 6, 257-261.

Riehl, R., Baensch, H.A., 1983. Aquarienatlas. Mergus Verlag für Natur- und Tierheimkunde Baensch, Melle, Germany.

Rochlitz, I., 2005. A review of the housing requirements of domestic cats (Felis silvestris ccitus) kept in the home. Appl. Anim. Behav. Sci. 93, 97-109.

Sachser, N., 1998. Of domestic and wild guinea pigs: Studies in sociophysiology, domestication, and social evolution. Naturwissenschaften 85, 307-317.

Schepers, F., Koene, P., Beerda, B., 2009. Welfare assessment in pet rabbits. Anim. Welfare 18, 477-485.

Schetini de Azevedo, C.S., Cipreste, C.F., Young, R.J., 2007. Environmental enrichment: A GAP analysis. Appl. Anim. Behav. Sci. 102, 329-343.

Schmid, I., Wechsler, B., 1997. Behaviour of Japanese quail (Coturnix japonica) kept in semi-natural aviaries. Appl. Anim. Behav. Sci. 55, 103-112.

Shepherdson, D., 1998. Tracing the Path of Environmental Enrichment in Zoos, In: Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), Second nature: Environmental Enrichment for Captive Animals, Smithsonian Institution Press, Washington, pp. 1-14.

Shepherdson, D.J., Carlstead, K., Mellen, J.D., Seidensticker, J., 1993. The influence of food presentation on the behaviour of small cats in confined environments. Zoo Biol. 12, 203-216.

Sherwin, C.M., 2004. The influences of standard laboratory cages on rodents and the validity of research data. Anim. Welfare 13, S9-S15.

Shyne, A., 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biol. 25, 317-337.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, Danio rerio. Biol. Rev. 83, 13-34.

Stauffacher, M., 1998. 15 Thesen zur Haltungsoptimierung im Zoo. Zool. Garten N.F. 68, 201-218.

Steiger, A., Gebhardt-Henrich, S.G. , 2008. Housing and breeding of pet and companion animals: a need for more welfare and research?, In: Hammond, E.P., Noyes, A.D. (Ed.), Housing: Socioeconomic, Availability & Development Issues, Nova Science Publishers, Inc.

Stolba, A., Woodgush, D.G.M., 1989. The Behavior of Pigs in a Semi-Natural Environment. Anim. Prod. 48, 419-425.

Swaisgood, R.R., Shepherdson, D.J., 2005. Scientific Approaches to Enrichment and Stereotypies in Zoo Animals: What's Been Done and Where Should We Go Next? Zoo Biol. 24, 499–518.

Swaisgood, R.R., Shepherdson, D.J., 2006. Environmental enrichment as a strategy for mitigating stereotypies in zoo animals: a literature review and meta-analysis, In: Mason, G., Rushen, J.

(Ed.), Stereotypic Behaviour in Captive Animals: Fundamentals and Applications for Welfare, 2nd ed, CAB International, Wallingford.

Swaisgood, R.R., White, A.M., Zhou, X., Zhang, G., Lindburg, D.G., 2005. How Do Giant Pandas (Ailuropoda melanoleuca) Respond to Varying Properties of Enrichments? A Comparison of Behavioral Profiles Among Five Enrichment Items. J. Comp. Psychol. 119, 325-334.

van Hoek, C.S., ten Cate, C., 1998. Abnormal Behavior in Caged Birds Kept as Pets. J. Appl. Anim. Welf. Sci. 1, 51-64.

Vascotto, S.G., Beckham, Y., Kelly, G.M., 1997. The zebrafish's swim to fame as an experimental model in biology. Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire 75, 479-485.

Veasey, J.S., Waran, N.K., Young, R.J., 1996. On comparing the behaviour of zoo housed animals with wild conspecifics as a welfare indicator. Anim. Welfare 5, 13-24.

Verga, M., Luzi, F., Carenzi, C., 2007. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm. Behav. 52, 122-129.

Waiblinger, E., König, B., 2004. Refinement of gerbil housing and husbandry in the laboratory. Anim. Welfare 13, S229-S235.

Wechsler, B., 2007. Normal behaviour as a basis for animal welfare assessment. Anim. Welfare 16, 107-110.

Williams, B.G., Waran, N.K., Carruthers, J., Young, R.J., 1996. The effect of a moving bait on the behaviour of captive cheetahs (*Acinonyx jubatus*). Anim. Welfare 5.

Williams, T.D., Readman, G.D., Owen, S.F., 2009. Key issues concerning environmental enrichment for laboratory-held fish species. Lab. Anim. 43, 107-120.

Wilson, S.F., 1982. Environmental Influences on the Activity of Captive Apes. Zoo Biol. 1, 201-209.

Wootton, R.J., Handisyde, N., Rowe, C., 2006. The effect of cover on food consumption and growth in two freshwater fish species used in experimental studies. Anim. Welfare 15, 309-313.

Würbel, H., 2001. Ideal homes? Housing effects on rodent brain and behaviour. Trends Neurosci. 24, 207-211.

Würbel, H., 2006. The Motivational Basis of Caged Rodents' Stereotypies, In: Mason, G., Rushen, J. (Ed.), Stereotypic Behaviour in Captive Animals: Fundamentals and Applications for Welfare, 2nd ed.

Würbel, H., Chapman, R., Rutland, C., 1998. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. Appl. Anim. Behav. Sci. 60, 69-81.

Young, R.J., 2003. Environmental Enrichment for Captive Animals. Blackwell Publishing Professional, Ames, Iowa, USA.

CHAPTER 1

Feeding enrichment in an opportunistic carnivore: the red fox

Feeding enrichment in an opportunistic carnivore: the red fox

Claudia Kistler[a], Daniel Hegglin[b], Hanno Würbel[c], Barbara König[a]

[a] Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland

[b] SWILD, Urban Ecology & Wildlife Research, Wuhrstrasse 12, Zürich, Switzerland

[c] Division of Animal Welfare and Ethology, Clinical Veterinary Sciences, University of Giessen, Giessen, Germany

ABSTRACT

In captive carnivores, species-specific behaviour is often restricted by inadequate feeding regimens. Feeding live prey is not feasible in most places and food delivery is often highly predictable in space and time which is considerably different from the situation in the wild. As a result, captive carnivores are often inactive, show little behavioural diversity and are prone to behavioural problems such as stereotypic pacing. Using artificial feeding devices to substitute for natural food resources is a way to address these problems. In a group of four red fox (*Vulpes vulpes*), we compared a conventional feeding method to four different methods through the use of feeding enrichment that were based on natural foraging strategies of opportunistic carnivores. Feeding enrichments consisted of electronic feeders delivering food unpredictable in time which were successively combined with one of three additional treatments: a self-service food box (allowing control over access to food), manually scattering food (unpredictable in space), and an electronic dispenser delivering food unpredictably both in space and time. The aim of administering feeding enrichment in this study was to stimulate appetitive (food searching) behaviour and to increase time spent feeding. Compared to conventional feeding, diversity of behaviour and overall activity were significantly enhanced in the presence of electronic feeders in all four foxes (EF > CON1=CON2, EF+SF > CON1=CON2, EF+MS > CON1 = CON2, EF+ED > CON1=CON2). Behavioural diversity was highest when the foxes had control over access to food (EF+SF), while the manual scattering of food (EF+MS) and the electronic dispenser (EF+ED) enhanced food searching behaviour. These results indicate that in opportunistic carnivores natural foraging and feeding behaviour can be stimulated by simple feeding enrichment strategies, and that foraging behaviour is stimulated most when food delivery is unpredictable both in space and time.

Keywords: Environmental enrichment, feeding enrichment, foraging, red fox, *Vulpes vulpes,* animal welfare, behavioural diversity, zoo

INTRODUCTION

There is increasing concern over the welfare of zoo animals, and animal welfare has become a central issue in zoo biology (Mason et al., 2007). Enclosures of wild animals in zoos and wildlife parks are often designed to mimic the animal's natural habitat (Robinson, 1998). Whether the illusion also satisfies the animals' behavioural needs, however, is often unclear. Zoo enclosures should provide wild animals with stimulating environments (Markowitz, 1982) to facilitate species-typical behaviour and to prevent abnormal behaviours. Consequently, 'naturalness' of behaviour and absence of abnormal behaviours are often used as proxy measures of good welfare. In particular, the more of its species-typical behavioural repertoire an animal can express, the better the enclosure is assumed to satisfy the animal's behavioural needs (Markowitz and LaForse, 1987; Shepherdson et al., 1993; Bashaw et al., 2003; Wechsler, 2007). 'The ability to perform most natural patterns of behaviour' is also one of the essential five freedoms listed by the Farm Animal Welfare Council (1993). However, others have argued that naturalness of behaviour is a poor measure of animal welfare (Dawkins, 2006). Indeed, not all behaviours seem essential for the well being of captive animals, as for example escaping from a predator, infanticide, or coping with natural obstacles (Hughes and Duncan, 1988; Veasey et al., 1996; Stauffacher, 1998; Dawkins, 2006).

Foraging behaviour is undeniably an essential part of all animals' lives. Under natural conditions, animals often spend most of their active time searching for food, as foraging is generally time consuming and the animals' activity phases are adapted to the foraging needs imposed by their habitats (Herbers, 1981; Shepherdson et al., 1993). In wild carnivores, the availability of food is typically unpredictable both in space and time, so they have to use specific skills to locate and exploit food resources. In contrast, feeding of captive carnivores is often predictable in space and time, and foraging is limited to food intake. Therefore, feeding enrichment based on the animals' natural foraging strategies may be crucial for the well being of captive carnivores (Lindburg, 1998; Bashaw et al., 2003).

Depending on their food spectrum, carnivores use a variety of foraging strategies. Foxes (*Vulpes sp.*) are opportunistic carnivores with a wide trophic niche. They exploit various food sources of which vegetables or fruit can make up a large part (Lucherini and Crema, 1994). Such food is persistently searched for, and sometimes skilfully exploited, especially by animals living in or near settlement

areas (Contesse et al., 2004). Thus, feeding enrichments may be particularly beneficial to opportunistic carnivores such as captive foxes.

Previous work in captive carnivores has used a variety of feeding enrichment strategies. Artificial moving prey elicited natural hunting behaviour in servals (*Felis serval*) and cheetahs (*Acinonyx jubatus*) (Markowitz and LaForse, 1987; Williams et al., 1996). In European wildcats (*Felis s. sylvestris*) automatic electronic feeders elicited natural hunting behaviour and prevented behavioural disturbances (Hartmann-Furter, 2000), whereas feeders which had to be opened by margays (*Leopardus wiedii*) did not elicit appetitive behaviour (Gusset et al., 2002). In tigers (*Panthera leo*), stereotypic behaviour was decreased by manipulable feeders (Jenny and Schmid, 2002). In lemurs (*Eulemur fulvus albifrons*, *Hapalemur griseus*) overall activity and locomotor behaviour was increased when food was offered in self-service food boxes (Sommerfeld et al., 2006). Hiding food in the enclosure reduced stereotypic behaviour in black bears *(Ursus americanus)* and increased searching behaviour in bush dogs (*Speothos venaticus*) (Carlstead et al., 1991; Ings et al., 1997).

Based on these findings, we studied four different feeding enrichments in a group of four red foxes housed in a near-to-natural outdoor enclosure to test for their effects on foraging and feeding activity and on behavioural diversity compared to conventional feeding. We hypothesised that the foxes would be most active and their behaviour most diverse, when food was presented unpredictably in space and time, and when it was most difficult (time consuming) to find. To test this hypothesis, we varied foraging demand and predictability of food in space and time by using electronic feeders (food unpredictable in time) either (i) alone or in combination with (ii) a self-service food box (unpredictable in time plus time-consuming manipulation), (iii) scattered and hidden food (unpredictable in time and space plus time-consuming), or (iv) an electronic dispenser (highly unpredictable in time and space plus time consuming). We predicted that general activity and behavioural diversity would increase from treatment (i) through to treatment (iv).

MATERIALS AND METHODS

Subjects and housing

The study was conducted between March and June 2005 in an outdoor enclosure at Langenberg wildlife park near Zürich, Switzerland. The enclosure was not accessible to visitors, but designed as a test enclosure for a new exhibit planned for foxes in the public part of the park.

The study subjects formed a group of four adult red foxes, two males and two females, which were unrelated to each other. All had been found as cubs in 2002, and had since lived together in the test enclosure. The outdoor enclosure spanned an area of 300m^2 of natural soil covered with grass and other plants. It was furnished with various structures such as resting places of different kinds, shrubs, hedges composed of small fir-trees, trees suitable for climbing, heaps of stones and earth, a wooden den and two artificial dens. Interference of dens by humans never occurred during the observation periods in order to provide the animals with a secure place to retreat.

Feeding treatments

The foxes were fed daily except on Saturdays when they were fasted. In all feeding treatments, the daily diet consisted of 400 g of meat (freshly killed rats or small pieces of meat), 200 g of fruits, and 200 g of dried dog food, raisins, sunflower seeds and nuts.

Five different feeding treatments were used throughout the study.

- In feeding treatment one (conventional, CON), all food was given at once always at the same time (0930 hours) and in the same place. Food supply was therefore predictable in time and space.

- In feeding treatment two (electronic feeders, EF), three computer-controlled electronic feeders (Hartmann-Furter, 2000) were located at three different sites (separated by approx. 10 meters) inside the enclosure. Each of the three feeders contained one third of the meat ration, two feeders additionally contained fruits, dried dog food, raisins, sunflower seeds and nuts. From the feeder with meat only, food was catapulted out of the food box when the shutter opened because the food was tied to an elastic cord fixed to the branch of a nearby tree. The other two feeders dropped the food down to the ground upon opening the shutter. Each feeder was opened once every day and

opening times were randomly distributed over the day between 1000 hours and 1800 hours. Food supply was therefore predictable in space, but unpredictable in time.

- In feeding treatment three (electronic feeders plus self-service food box, EF+SF), a wooden self-service food box was installed in addition to the three electronic feeders. The box was held 80 cm above the ground by a rope attached to a pole, and was filled with nuts, dried dog food and sunflower seeds (25% of the daily diet). Upon manipulating a handle, the food inside the box fell through a small whole to the ground. The remaining food was distributed across the three electronic feeders, which were operated as described above. Therefore, food supply was again predictable in space, with 75% of the food supply being unpredictable in time, and 25% of the food supply being accessible by performing a specific manipulation.

- In feeding treatment four (electronic feeders plus manually scattered and hidden food, EF+MS), 100% of nuts, dried dog food, raisins and sunflower seeds, 20% of meat, and 50% of fruits were manually scattered and hidden in the enclosure. 80% of the meat and 50% of the fruits were distributed across the three electronic feeders, which were operated as described above. Thus, about half of the food supply was predictable in space, but unpredictable in time, while the other half was unpredictable both in space and time and, therefore, required additional foraging time.

- In feeding treatment five (electronic feeders plus electronic dispenser, EF+ED), an electronic dispenser was installed in addition to the three electronic feeders. The dispenser consisted of a plastic tub, with a distributor placed inside to partition the food into small portions and an analogue timing device. The mechanism activating the distributor was started by the timer. On activation, the distributor released a small amount of food, which fell onto a fast rotating disk and from there was dispersed within a radius of about six metres from the dispenser. The device was filled with nuts, dried dog food and sunflower seeds (25% of the daily diet). It was placed at the same location as the self-service food box and food was provided in eight portions every day at times that were different from the times when the electronic feeders released food. The electronic feeders were filled with the remaining 75% of meat, fruit and raisins. This treatment provided the highest degree of temporal and spatial unpredictability, and like treatment four (EF+ED) required additional foraging time.

Throughout the study, food was placed inside the different devices half an hour before observation started in the morning.

Experimental design and data recording

The foxes were presented with the four different feeding enrichments in a fixed order: EF, EF+SF, EF+MS, and EF+ED, and conventional feeding was presented before (CON1) and after (CON2) the feeding enrichments. Each of the six treatments lasted two weeks. The first week was used to habituate the foxes to the new feeding treatment. During the second week, data were collected on five days for four hours each day from 1000 to 1100 hours, 1200 to 1300 hours, 1400 to 1500 hours and from 1600 to 1700 hours. Behavioural data were collected by direct observations from an elevated hide with the aid of binoculars. The foxes were used to the observer's presence on the hide.

Prior to the study, *ad libitum* sampling over a period of 14 days served to establish a detailed ethogram that was complemented by information from the literature (Tembrock, 1957, 1982). All behaviours were grouped into one of eleven functional categories: exploration, monitoring, food searching, food-acquisition, feeding, resting, escape behaviour, other forms of locomotion (walking, trotting, gallop and jumping), comfort behaviour, social behaviour (socio-positive and socio-negative), and social behaviour during feeding bouts. Stereotypic behaviour or extended aggression did not occur during these pilot observations.

During the main study, focal animal sampling was used to assess behavioural diversity, while general activity was assessed using scan sampling. Thus, during each observation hour, each fox was continuously observed during six consecutive periods of 2.5 minutes (in total 15 minutes), whereby each behaviour was recorded only once. The order in which the four individuals were observed was kept the same throughout the day but was randomised between days. In addition, instantaneous observations at 2.5 min intervals were conducted to scan general activity. Activity was defined as all behaviours except resting and sleeping.

Data analyses

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

*H = - Σ (p$_i$*ln p$_i$),*

where p$_i$ is the relative abundance of each functional category, calculated as the proportion of behavioural elements of a given functional category to the total number of behavioural elements of all functional categories: n$_i$ / N. The index was calculated per feeding treatment and per individual. It increases with increasing numbers of functional categories, and as the relative representation of each functional category becomes more even. Lower indices represent lower behavioural diversity. To quantify activity, the number of active behaviours was summed up over total observation time per feeding treatment and per individual, and the proportion of active behaviour was calculated. To establish time budgets, behaviours of functional categories were summed up per hour and per feeding treatment for all individuals. Functional categories were combined into the following main categories: Exploration (locomotion, exploring, monitoring), food (food searching, food acquisition, feeding, social food), other (comfort, escape, and social behaviour), and resting. Overall mean percentage of main categories was calculated per feeding treatment.

A Friedman-test (Zar, 1999) was used to test for significant differences in individual behaviour between the six feeding treatments for all parameters. In the case of overall significance (p≤0.05), post-hoc test after Conover (1980) was used to compare single feeding treatments. SPSS (Version 13.0 for Windows) was used for statistical tests.

RESULTS

Effects of feeding enrichment on behavioural diversity

Diversity of behaviour differed significantly between feeding treatments (χ^2 = 15.571, p = 0.001, df = 5, n = 4; Fig. 1), with the lowest indices found during the first conventional feeding. Behavioural diversity was increased during all enriched feeding treatments, and declined again during the final conventional

feeding. Peak median diversity occurred during the treatment allowing for self manipulation of access to food (EF+SF). Post-hoc comparisons revealed that all feeding enrichments differed significantly from both the conventional feeding treatment presented in the beginning and at the end of the experiment (EF > CON1 = CON2, EF+SF > CON1 = CON2, EF+MS > CON1 = CON2, EF+ED > CON1 = CON2). Furthermore, the diversity index in the EF+SF treatments was significantly higher than in the three other feeding enrichments (all differences $p \leq 0.05$). All four individuals showed the same pattern over the course of the experiment.
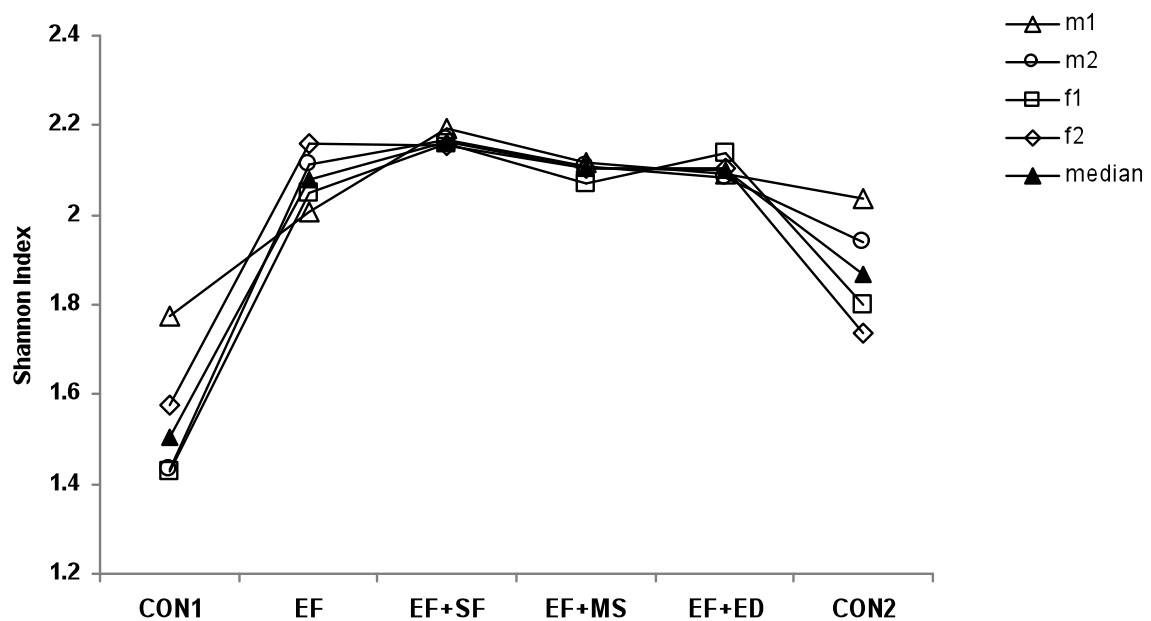
Figure 1. Individual behavioural diversity during conventional feeding and different feeding enrichment treatments. Values of Shannon diversity index of four red foxes (two males, m1, m2, and two females, f1, f2) and overall median are shown. Lower indices represent lower behavioural diversity, and higher indices represent higher behavioural diversity. EF = electronic feeders, SF = self-service food box, MS = manually scattered and hidden food, ED = electronic dispenser. CON1 and CON2 = conventional feeding before and after feeding enrichments, respectively.

Effects of feeding enrichment on activity

Overall activity differed significantly between the different feeding treatments (χ^2 = 16, p = 0.001, df = 5, n = 4; Fig. 2), with the lowest activity found during the first conventional feeding treatment. Activity increased during feeding enrichment treatments, and decreased again during the final conventional

feeding. Peak median activity occurred during the treatment when food presentation was most unpredictable in time and space (EF+ED). Post-hoc comparisons revealed that all feeding enrichments differed significantly from both the conventional feeding treatment at the beginning and at the end of the experiment (EF > CON1 = CON2, EF+SF > CON1 = CON2, EF+MS > CON1 = CON2, EF+ED > CON1 = CON2). Furthermore, activity during EF+SF was significantly lower than during EF+MS and EF+ED (EF+SF < EF+MS = EF+ED).
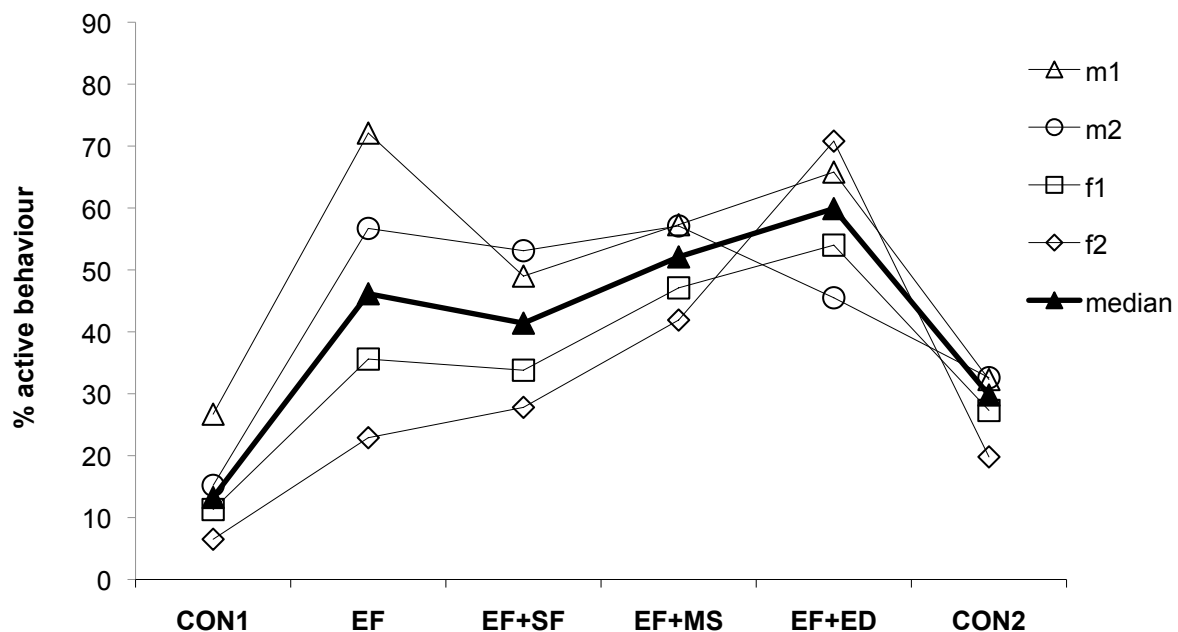

Figure 2. Activity (% of observation time) of four red foxes (two males, m1, m2, and two females, f1, f2) during conventional feeding and different feeding enrichment treatments, EF = electronic feeders, SF = self-service food box, MS = manually scattered and hidden food, ED = electronic dispenser. CON1 and CON2 = conventional feeding before and after feeding enrichments, respectively.

Figure 3 shows the activity budget of the group during feeding treatments. During CON1 and CON2, mean time spent active was 14% and 26%, respectively. Exploration and food related behaviour increased during all four enriched feeding treatments. Mean percentage of exploration ranged between 21% during EF+SF and 29% during EF and EF+ED. Mean percentage of food related behaviour increased continuously from 10% during EF to 22% during EF+ED. Other behaviours

(comfort, flight and social behaviour) increased during EF, EF+SF, and EF+MF, but decreased again during EF+ED.
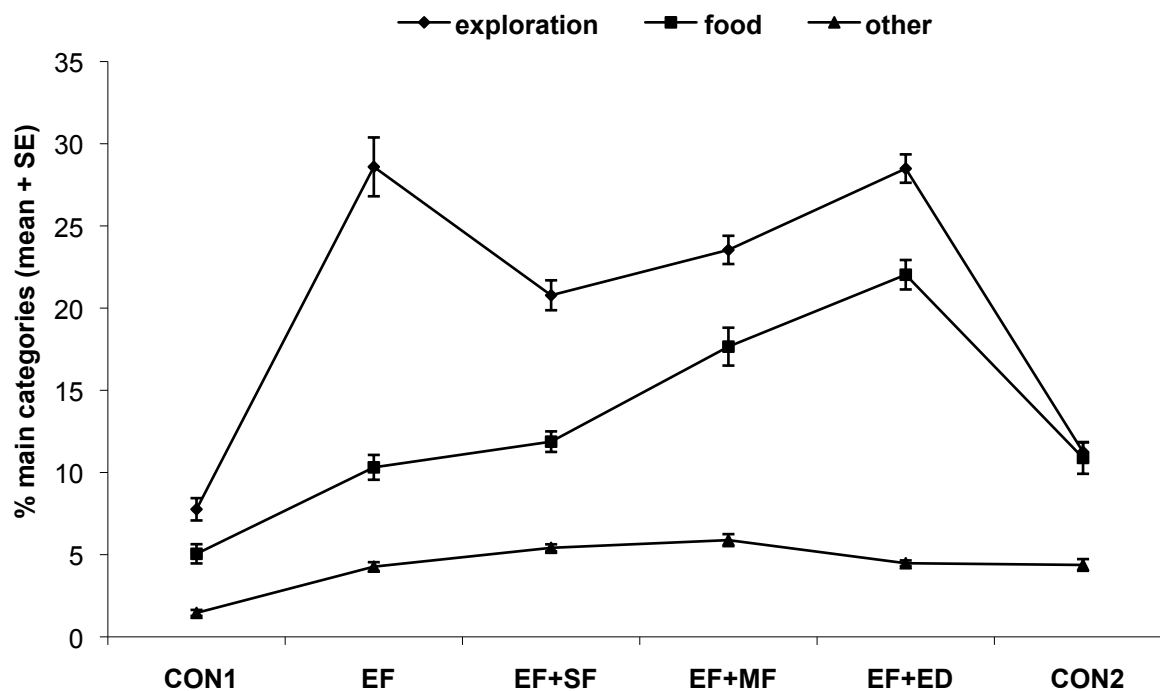

Figure 3. Activity budget (overall mean + SE) of the fox group during conventional feeding and feeding enrichment treatments. Main categories are exploration (locomotion, exploration, monitoring), food (food searching, food acquisition, feeding, social food), other (comfort, escape, and social behaviour). EF = electronic feeders, SF = self-service food box, MS = manually scattered and hidden food, ED = electronic dispenser. CON1 and CON2 = conventional feeding before and after feeding enrichments, respectively.

DISCUSSION

Consistent with our predictions, all four feeding enrichments significantly enhanced individual behavioural diversity and activity of the four red foxes compared to a conventional feeding treatment. Our findings indicate that any kind of temporal and/or spatial unpredictability in the presentation of food has a stimulating effect on the foxes' behaviour.

In all enriched feeding treatments, behavioural diversity was increased in all functional categories, except resting. Behavioural diversity was highest when the foxes were able to manipulate access to food (EF+SF). However, three of the four foxes showed the highest absolute numbers of behavioural elements when the spatial and temporal unpredictability of food was highest (EF+ED). Unpredictability of feedings can cause enhanced abnormal or agonistic behaviour prior to feedings (Waitt and Buchanan-Smith, 2001). It is assumed that this is connected with unreliable signals caused by

caretakers. As in this study feeding times were not linked to the caretakers' presence no external unreliable signals occurred. The foxes never showed any signs of behavioural disturbances such as stereotypies or extended aggression. Thus, all feeding enrichments induced higher activity and a greater diversity of behaviour without causing behavioural problems. Since the diurnal pattern of activity of wild red foxes varies greatly depending on the diurnal pattern of food availability (Zabel and Taggart, 1989; Cavallini and Lovari, 1991; Ricci et al., 1998) and human activity (Lucherini et al., 1995; Gloor, 2002), feeding enrichment during daylight hours appears to be an adequate form of enrichment in red foxes.

During both the initial and final conventional feeding treatment, resting was high in all four foxes. Providing food once daily at a particular time is common in zoos and wildlife parks due to operating schedules (Carlstead, 1991; Carlstead et al., 1991; Shepherdson et al., 1993; Hartmann-Furter, 2000). In our study animals, this treatment hardly stimulated any food searching behaviour. General activity was considerably higher in all feeding enrichments. While the type of feeding enrichment did not affect levels of general activity, however, the proportion of food related behaviour such as food searching or food acquiring increased the most during the feeding treatment with the highest spatial und temporal unpredictability (EF+ED). Here, the foxes repeatedly browsed the enclosure. Furthermore, food items such as nuts, sunflower seeds and dog food are of small size, and finding them in the natural vegetation is time consuming. Along with food related behaviour there was an increase in exploratory behaviour such as locomotion and monitoring behaviour. The least amount of exploratory behaviour was elicited when the foxes had control over access to food items concentrated in a self-service food box (EF+SF). Although food related behaviour such as manipulative food acquiring behaviour increased, the foxes were less active compared to treatments when food was provided spatially and temporally unpredictable (EF+MS, EF+ED).

Because the four foxes belonged to one group, we could not randomise the order of feeding enrichments across individuals. Therefore, the trend towards increased exploration and food related behaviour with increasing unpredictability of food presentation is confounded by test order. However, the foxes adapted quickly to feeding conditions during the habituation weeks before data were collected and no differences were found in the foxes' responses to the first and last conventional feeding regime. Therefore, it is unlikely that the differences between feeding treatments are explained

by test order effects alone. Furthermore, the four subjects may have influenced each other in their behaviour. However, foxes are solitary foragers and do not monopolise food resources (Macdonald, 1988). Therefore, our findings are likely to generalise beyond this specific study group.

In accordance with other studies on feeding enrichment in captive carnivores (e.g. Carlstead et al., 1991; Hartmann-Furter, 2000; Bashaw et al., 2003; Sommerfeld et al., 2006), our results indicate that feeding enrichments based on natural activity patterns and feeding strategies can effectively stimulate species-specific behaviour. Increased activity and behavioural diversity induced by feeding enrichments are also likely to reflect improved well being, although independent evidence based on more direct measures of well-being is needed to confirm this.

REFERENCES

Bashaw, M.J., Bloomsmith, M.A., Marr, M.J., Maple, T.L., 2003. Feeding enrichment experiment with captive large felids. Zoo Biol. 22, 189–198.

Carlstead, K., 1991. Husbandry of the Fennec fox *Fennecus zerda*: Environmental conditions influencing stereotypic behaviour. Int. Zoo Yrbk 30, 202-207.

Carlstead, K., Seidensticker, J., Baldwin, R., 1991. Environmental Enrichment for Zoo Bears. Zoo Biol. 10, 103-116.

Cavallini, P., Lovari, S., 1991. Environmental factors influencing the use of habitat in the red fox, *Vulpes vulpes*. J. Zool. 223, 323-339.

Conover, W.J., 1980. Practical nonparametric statistics. 2nd ed. New York: Wiley.

Contesse, P., Hegglin, D., Gloor, S., Bontadina, F., Deplazes, P., 2004. The diet of urban foxes
(*Vulpes vulpes*) and the availabilty of anthropogenic food in the city of Zurich, Switzerland.
Mamm. Biol. 69, 81-95.

Dawkins, M.S., 2006. A user's guide to animal welfare science. TrendsEcolEvol 21, 77-28.

Farm Animal Welfare Council, 1993. Second report on priorities for research and development in farm
animal welfare. FAWC, Ministry of Agriculture, Fisheries and Food, Tolworth (Now DEFRA,
London).

Gloor, S., 2002. The rise of urban foxes (*Vulpes vulpes*) in Switzerland and ecological and
parasitological aspects of a fox population in the recently colonised city of Zurich. Dissertation
Universität Zürich.

Gusset, M., Burgener, N., Schmid, H., 2002. Wirkung einer aktiven Futterbeschaffung mittels
Futterkisten auf das stereotype Gehen und den Glukokortikoidspiegel von Margays,
Leopardus wiedii, im Zürich Zoo. Zool. Gart. N.F. 72, 245-262.

Hartmann-Furter, M., 2000. A species-specific feeding technique for european wildcats (*Felis s.
sylvestris*) in captivity. Säugetierkd. Info. 4, 567-575.

Herbers, J.M., 1981. Time resources and laziness in animals. Oecologia 49, 252-262.

Hughes, B.O., Duncan, I.J.H., 1988. The notion of ethological 'need', models of motivation and animal
welfare. Anim. Behav. 36, 1696-1707.

Ings, R., Waran, N.K., Young, R.J., 1997. Effect of wood-pile feeders on the behaviour of captive bush
dogs (*Speothos venaticus*). Anim. Welfare 6, 145-152.

Jenny, S., Schmid, H., 2002. Effect of feeding boxes on the behavior of stereotyping amur tigers
(*Panthera tigris altaica*) in the Zurich Zoo, Zurich, Switzerland. Zoo Biol. 21, 573–584.

Lindburg, D.G., 1998. Enrichment of captive mammals through provisioning. In: Shepherdson, D.J.,
Mellen, J.D., Hutchins, M., (Eds.), Second nature: Environmental Enrichment for Captive
Animals, Smithsonian Institution Press, Washington, pp 262-276.

Lucherini, M., Crema, G., 1994. Seasonal variation in diet and trophic niche of the red fox in an alpine
habitat. Z. Säugetierkd. 59, 1-8.

Lucherini, M., Lovari, S., Crema, G., 1995. Habitat use and ranging behaviour of the red fox (*Vulpes
vulpes*) in a Mediterranean rural area: Is shelter availability a key factor? J. Zool. 237, 577-
591.

Macdonald, D.W., 1988. Running with the fox. Unwin Hyman.

Markowitz, H., 1982. Behavioural Enrichment in the Zoo. Van Nostrand Reinhold, New York.

Markowitz, H., LaForse, S., 1987. Artificial prey as behavioral enrichment devices for felines. Appl. Anim. Behav. Sci. 18, 31-43.

Mason, G., Clubb, R., Latham, N., Vickery, S., 2007. Why and how should we use environmental enrichment to tackle stereotypic behaviour? Appl. Anim. Behav. Sci. 102, 163-188.

Ricci, S., Colombini, I., Fallaci, M., Scoccianti, C., Chelazzi, L., 1998. Arthropods as bioindicators of the red fox foraging activity in a Mediterranean beach-dune system. Journal of Arid Environments 38, 335-348.

Robinson, M.H., 1998. Enriching the lives of zoo animals, and their welfare: where research can be fundamental. Anim. Welfare 7, 151-175.

Shannon, C.E., Weaver, W., 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

Shepherdson, D.J., Carlstead, K., Mellen, J.D., Seidensticker, J., 1993. The influence of food presentation on the behaviour of small cats in confined environments. Zoo Biol. 12, 203-216.

Sommerfeld, R., Bauert, M., Hillmann, E., Stauffacher, M., 2006. Feeding enrichment by self-0perated food boxes for white-fronted lemurs (*Eulemur fulvus albifrons*) in the Masoala exhibit of the Zurich Zoo. Zoo Biol. 25, 145-154.

Stauffacher, M., 1998. 15 Thesen zur Haltungsoptimierung im Zoo. Zool. Gart. N.F. 68, 201-218.

Tembrock, G., 1957. Das Verhalten des Rotfuchses. Handbuch der Zoologie 8.

Tembrock, G., 1982. Zum Verhalten des Rotfuchses *Vulpe vulpes* (L., 1758). Beitr. Jagd-Wildforsch. 12, 37-43.

Veasey, J.S., Waran, N.K., Young, R.J., 1996. On comparing the behaviour of zoo housed animals with wild conspecifics as a welfare indicator. Anim. Welfare 5, 13-24.

Waitt, C., Buchanan-Smith, H.M., 2001. What time is feeding? How delays and anticipation of feeding schedules affect stump-tailed macaque behavior. Appl. Anim. Behav. Sci. 75, 75-85.

Wechsler, B., 2007. Normal behaviour as a basis for animal welfare assessment. Anim. Welfare 16, 107-110.

Williams, B.G., Waran, N.K., Carruthers, J., Young, R.J., 1996. The effect of a moving bait on the behaviour of captive cheetahs (*Acinonyx jubatus*). Anim. Welfare 5.

Zabel, C.J., Taggart, S.J., 1989. Shift in red fox, *Vulpes vulpes*, mating system associated with El Niño in the Bering Sea. Anim. Behav. 38, 830-838.

Zar, J.H., 1999. Biostatistical analysis. 4th ed. Upper Saddle River, Prentice Hall, New Jersey.

CHAPTER 2

Structural enrichment and enclosure use in an opportunistic carnivore: the red fox (*Vulpes vulpes*)

Animal Welfare (2010), 19: 391-400

Structural enrichment and enclosure use in an opportunistic carnivore: the red fox (Vulpes vulpes)

Claudia Kistler[a], Daniel Hegglin[b], Hanno Würbel[c], Barbara König[a]

[a] Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland

[b] SWILD, Urban Ecology & Wildlife Research, Wuhrstrasse 12, Zürich, Switzerland

[c] Division of Animal Welfare and Ethology, Clinical Veterinary Sciences, University of Giessen, Giessen, Germany

ABSTRACT

An increasing number of zoos keep their animals in naturally looking enclosures, but whether or not the species' ecological and behavioural needs are adequately met is often unclear. For species suffering predation in the wild, structural enrichment in captivity can play a crucial role in connection with enclosure use. Firstly, we examined the effectiveness of structural enrichment in modifying enclosure use in an opportunistic carnivore, the red fox (Vulpes vulpes). In a test enclosure, we placed both long wooden structures and cover structures that simulated natural habitat in predetermined sectors. A group of four foxes were exposed to four treatments: (i) structural enrichment in location 1 (LOC1s); (ii) structural enrichment in location 2 (LOC2); (iii) structural enrichment removed (REM); and (iv) structural enrichment again in location 1 (LOC1e). Sectors containing long wooden structures were significantly preferred to the rest of the enclosure. Sector use was selectively shifted to those sectors where cover structures were present. Structural enrichment had no significant effect on activity. Secondly, in a new outdoor enclosure, we compared the use of sectors with cover or elongated structures with that of corresponding sectors without structures. All individuals showed a significant preference for sectors containing structures. In the course of the three-week observation period, there was a significant decline in the preference for structures and a significant increase in activity (week 1 < week 2 = week 3). These results suggest that in medium-sized carnivores, structural enrichment is beneficial when natural features and their netlike distribution over the habitat are simulated.

Keywords: Animal welfare, environmental enrichment, red fox, structural preference, Vulpes vulpes, zoo

INTRODUCTION

Structural enrichment can enhance the complexity of captive environments (Maple & Perkins 1996), but in contrast to feeding enrichment, structural enrichment is rarely applied in zoos (Schetini de Azevedo *et al* 2007). Nevertheless, an increasing number of zoo exhibits are designed to mimic the animal's natural habitat (Robinson 1998). Outdoor enclosures offer spatial variation due to natural elements dividing the enclosure in various sections, and temporal variation due to daily and seasonal changes (Hutchins *et al* 1984). Richly structured enclosures provide animals with a variety of stimuli and structure space both horizontally and vertically (Hediger 1942, Maple & Perkins 1996). It is often thought that environmental complexity is highest in natural-looking enclosures thereby allowing the animals to show their natural behaviour (Hutchins *et al* 1984). However, even in natural-looking enclosures, the placement of structures needs to be well considered and to be adjusted to the animals' use of structures (Hutchins *et al* 1984). Moreover, functional substitution of natural elements with structures that serve the same function has been applied successfully in the past (Mellen *et al* 1981, Markowitz 1982, Robinson 1998). Various carnivore species kept in captivity are highly exploratory, and activities including foraging, territorial, social and play behaviour can cover a considerable part of the animal's daily time budget (Poole 1992). Enclosures need therefore to be furnished such that the ecological and behavioural needs of the animals are met in order to guarantee their well-being (Hughes & Duncan 1988, Broom 2007).

Previous studies showed that structural enrichment reduces abnormal behaviour or elicits natural behaviour. In spectacled bears (*Tremarctos ornatus*) pacing and motionless sitting were reduced and general activity increased by adding climbing structures (Renner & Lussier 2002). In a study on clouded leopards (*Neofelis nebulosa*), tight ropes elicited natural climbing behaviour (Hartmann & Schiess 1997), and Indian leopards (*Panthera pardus*) performed less stereotypic behaviour when the outdoor enclosure was furnished with natural features (Mallapur & Chellam 2002). In captivity, cover is an important feature for species that suffer high predation risks and whose natural habitats are rich in cover. Thus, adding cover structures to the cages reduced stereotypic behaviour in bank voles (*Clethrionomys glareolus*), gerbils (*Meriones unguiculatus*) and laboratory mice (Ödberg 1987, Wiedenmayer 1997, Würbel *et al* 1998). Japanese quails (*Coturnix japonica*) showed less flight responses to a flight inducing stimulus when cages were enriched with cover structures (Buchwalder & Wechsler 1997). Stereotypic running in fennec fox (*Fennecus zerda*) was elicited through noise from

keepers and visitors. The lack of space and hiding places was regarded as the main cause for this abnormal behaviour (Carlstead 1991). Gusset (2005) hypothesised that hiding as a coping strategy can reduce stress in margays (*Leopardus wiedii*) showing stereotypic behaviour. In farmed foxes (*Vulpes vulpes, Alopex lagopus*), abnormal behaviour was attributed to the barren cages and to the fear of humans (Nimon & Broom 2001). Red foxes (*Vulpes vulpes*) have evolved in an environment in which they suffered predation from larger canid or felid species and humans. Their habitats include cover-rich areas, and fox trails are frequently found along natural structures (Lucherini *et al* 1995, Adkins & Stott 1998, White *et al* 2006). Therefore, captive foxes may benefit from structural enrichment that takes into account the environment within which they have evolved and become adapted to.

In the first part of this study, two experiments on structural enrichment were conducted with a group of four foxes housed in a near-to-natural outdoor enclosure. We used two types of enrichment, long wooden structures and cover structures. Long wooden structures were designed as substitutes for hedges or walls and provided the foxes the possibility to move along a structure while ranging in the enclosure. Cover structures were designed as substitutes for scrub or thicket through which the foxes could slip while ranging in the enclosure. The experimental structures were placed in predetermined sectors, shifted in location, and removed from the enclosure. We tested the efficiency of these structural enrichments in influencing enclosure use and enhancing activity. Our prediction was that the foxes' preference for sectors would shift depending on the location of the structural enrichment. Furthermore, we expected the activity to be higher in treatments with structural enrichment than in those without. Thus, the aim of the first part of the study was to assess the preferences for structural components in a species that lives in cover-rich habitats.

In the second part of this study, we conducted an experiment on structure use in a newly built natural-looking enclosure into which the same group of foxes were transferred. Various structures were placed in the enclosure to provide cover. We tested for the foxes' preferences among eleven of these structures in comparison to virtual structures that were assigned to each of the eleven real structures. We expected the foxes to prefer the real structures over the virtual structures. We also expected the activity to increase in the course of the observation period. The aim of the second part of the study was to test whether the structures in the new enclosure were used by the foxes, thereby serving their purpose.

MATERIALS AND METHODS

Experiment 1 - Experiments in test enclosure

Subjects and housing

This study was conducted in an outdoor enclosure at Langenberg Wildlife Park near Zürich, Switzerland. The enclosure was not accessible to visitors, but designed as a test case for a new exhibit to be planned for foxes in the public part of the park. We refer to it therefore as test enclosure.

The study subjects formed a group of four unrelated adult red foxes, two males and two females. All had been found as cubs in 2002 and thereafter lived together in the test enclosure. None of the females reared cubs during the study period. The outdoor enclosure spanned an area of $300m^2$ of natural soil covered with grass and other plants. It was furnished with various structures, such as a variety of resting places, shrubs, hedges composed of small fir-trees, trees suitable for climbing, heaps of stones and earth, a wooden den and two artificial dens. Human intervention at the den never occurred during the observation periods in order to provide the animals with a secure place to retreat. Behavioural observations and the structural enrichment experiments were carried out from July 2004 to October 2004, when the foxes were two years of age. The daily food intake consisted of 400 g of meat and 200 g of fruit, nuts and raisins, with food provided by electronic feeders (Hartmann-Furter 2000), a self-service food box and by manually scattering and hiding food to simulate the situation in the wild. For a detailed description of this temporally and spatially unpredictable feeding method that stimulated natural foraging behaviour, see Kistler *et al* (2009).

Structural enrichment with wooden structures

The foxes were presented with four consecutive treatments. At the start, in treatment one (wLOC 1s), four long wooden structures were placed at four different sites in the enclosure. These structures had to be placed at the flat part of the enclosure (Figure 1, top). The structures consisted of four wooden walls: two that measured 600 x 2 x 80 cm (length x breadth x height) and two that were 600 x 50 x 80 cm. In treatment two (wLOC 2), each of the four long wooden structures were moved from the initial sites to new sites. The distance to the first location was approximately five meters. In treatment three (wREM), the four long wooden structures were removed from the enclosure and in treatment four (wLOC 1e), the four long wooden structures were replaced at the same location as in wLOC 1s.

Structural enrichment with cover structures

After the experiment with the long wooden structures, the foxes were presented with four different treatments concerning use of cover (Figure 1, middle).

In treatment one (cLOC 1s), nine wooden cover structures were placed at nine different sites in the enclosure (Figure 1, middle). Cover structures were 80 x 50 cm (length x breadth). Six were artificial solid wooden passages, and three were hollow stumps through which the foxes could slip. Of the three hollow stumps only two were available to the foxes at any given time, and one was blocked with wooden planks. In total, eight cover structures were accessible to the foxes.

In treatment two (cLOC 2), each of the six wooden passages and one hollow stump were moved to a new site. The distance to the first location was approximately five meters. The hollow stump that was blocked in treatment one was now opened and the other one blocked with wooden planks. In treatment three (cREM), the six wooden passages were removed from the enclosure and all hollow stumps were blocked with wooden planks.In treatment four (cLOC 1e), the six wooden passages and one hollow stump were placed at the same locations as in LOC 1s, and the same hollow stump, which had been accessible in treatment one, was re-opened.

Experimental set-up and data recording

To record the pattern of structure use, the enclosure was divided into 211 sectors, each measuring 1.25 m x 1.25 m. In the experiment on long wooden structures, 20 sectors contained experimental structures in treatment one and four, and 16 contained experimental structures in treatment two. In the experiment on cover structures, nine sectors contained experimental structures in treatment one, two, and four. Sectors containing no experimental structures were referred to as the rest of enclosure.

Each of the four treatments in both manipulations lasted for a period of one week. After three days of habituation to the structural enrichment, behavioural data were collected on day four, five and seven for five hours a day between 1200h and 2030h. After the last observation bout on day seven, experimental structures were manipulated according to the experimental setup. Behavioural data were collected by direct observations from an elevated hide with the aid of binoculars. The foxes were used to the observer's presence on the hide. For definitions of behaviours recorded, see Kistler *et al* (2009).

Activity of the individuals and use of structures were recorded using scan sampling at 2.5-min intervals (Altmann 1974). Activity was defined as all behaviours except resting and sleeping.

Data analysis

To assess sector use in the four treatments, the number of sectors used was summed up over total observation time per treatment and individual (15 h), and the ratio s1/s2 calculated where s1 refers for sectors that contained experimental structures in treatment one and four, respectively, and s2 sectors that contained experimental structures in treatment two. To assess the preference for sectors containing experimental structures over the rest of the enclosure (R), the ratio (s1 + s2)/R was calculated per treatment and per individual.

To determine activity, the number of active behaviours was summed up over total observation time per treatment and per individual, and the proportion of active behaviour was calculated.

A Friedman-test (Zar 1999) was used to test for significant differences in individual behaviour between the four treatments for all parameters. In the case of overall significance ($P \leq 0.05$), *post hoc* tests, after Conover (1980), were used to compare single treatments. SPSS (Version 13.0 for Windows) was used for all statistical tests.
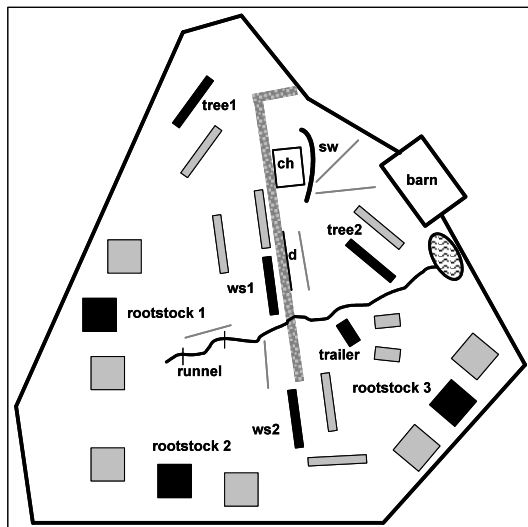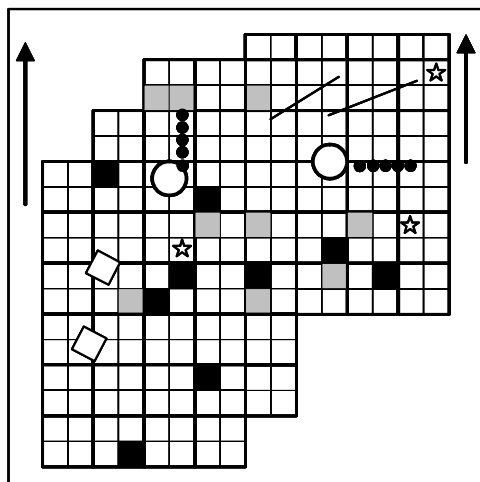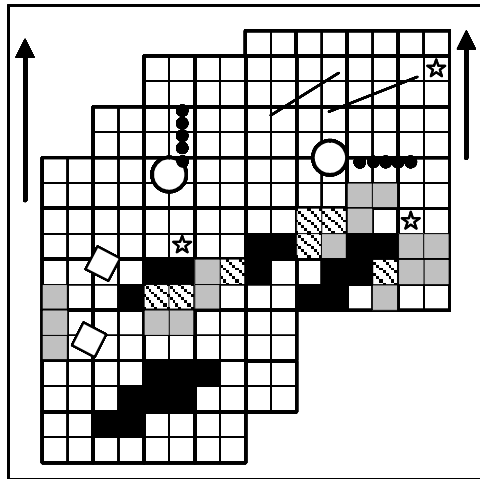
Figure 1: Test enclosure and quadratic sectors during experiments with long wooden structures (top) and cover structures (middle). Black squares: sectors containing experimental structures in treatment 1 and 4 (LOC 1s and LOC 1e), grey squares: sectors containing experimental structures in treatment 2 (LOC2), dashed squares: sectors that contained experimental structures in treatment 1, 2 and 4, white sectors: rest of enclosure (R), open circles: artificial dens, open rectangles: doghouse and wooden den, black circles: hedges, black lines: felled trees, stars: electronic feeders. The black arrows indicate the slope in the enclosure. New enclosure (bottom) with 11 real (black) and corresponding virtual (grey) structures. ch: chicken house, d: declivity, sw: stone wall, ws1 and ws2: wooden stacks.

Experiment 2 - Structure use in large new enclosure

Subjects and housing

In September 2007, all four individuals were transferred to a newly built enclosure in the Langenberg Wildlife Park. The natural-looking enclosure spanned 4000m^2 and had been designed as an agricultural landscape. It was richly furnished with elements that can be found on a farm, such as a chicken house, a trailer, an orchard, a runnel (that ran vertically through the centre of the enclosure from the upper to the lower side), wooden stacks, rootstocks, felled trees, and various shrubs. There were two artificial dens. The structures were arranged, netlike, such that they offered cover at various points when the foxes ranged in the enclosure. Visitors had access to the enclosure only on its lowest side where they could also enter a barn. The barn had open windows on the side that faced the enclosure offering a view over the whole enclosure. As electronic feeders were not yet operational during the study period, food was manually scattered and hidden by the observer before observations started throughout the entire enclosure except in the experimental areas designed to test the use of real or virtual structures. Food consisted of meat or rats, fruit, nuts, dried dog food, sunflower seeds and raisins.

Experimental set-up and data recording

The following eleven structures were used during behavioural observations besides the chicken house, six fruit trees, and newly planted bushes: two wooden stacks (6 x 2–3 m; length x height), two tree trunks of 8 m length and 1–2 m diameter, a stone wall of 12 x 1.5 m (length x height), a section of the declivity of 8 m length, including the second artificial den, a section of the runnel of 5 m length, the trailer of 6 x 2 x 1 m (length x breadth x height) and three big rootstocks with a diameter of 4 m and height of 2 m (Figure 1, bottom). To each of these structures two virtual structures of similar size were assigned in a distance of approximately 4 m to the real structure. The edges of these virtual structures were marked with sticks resulting in a corresponding area containing no structures only grass. Due to space restrictions, only one virtual structure each was assigned to the two felled trees and the declivity.

Data recording lasted three weeks starting one day after the transfer of the foxes. Data were collected for three days a week and four hours a day between 1300h and 1830h. Behavioural data were collected by direct observations with the aid of binoculars from the visitor's barn from where the whole

enclosure could be overlooked. During data collection, public access to the enclosure was not permitted. The same ethogram was used as in the experiments in the test enclosure. To record activity and use of real and virtual structures, scan sampling at 2.5-min intervals (Altmann 1974) was used.

Data analysis

In order to obtain a preference value for structure use, the Jacobs' preference index (Jacobs 1974) was calculated as:

$J = (r-P) / [(r+P) - 2rP]$

where *r* is the ratio of the number of real structures used to the number of all real and virtual structures used, and *P* is the available proportion of each structure. Thus, for structures with two virtual structures (*P* = 0.333), and for structures with one virtual structure (*P* = 0.5). The index ranges between +1 for maximum preference, and -1 for maximum avoidance. To examine preference for real structures over the whole observations period (36 hours) the index was calculated per individual and per structure. 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, where n is the number of structures used in the analysis).

To examine development in structure use over time, we calculated the Jacobs' preference index for all structures per individual separately for the first two weeks and the third week. Activity of the foxes was rather low during the first week, therefore data for the first and second week were pooled. For comparison of the two periods we used a Wilcoxon Signed Ranks Test (Zar 1999).

To measure activity, the number of active behaviours was totalled per week and per individual, and the mean proportion of active behaviour was calculated. A Friedman-test (Zar 1999) was used to test for significant differences in individual activity between the three weeks. In the case of overall significance (*P* ≤ 0.05), post-hoc tests after Conover (1980) were used to compare single treatments. SPSS (Version 13.0 for Windows) was used for statistical tests.

RESULTS

Effects of structural enrichment on spatial behaviour in the test enclosure

The foxes increased the use of the long wooden structures during treatment four (wLOC 1e), when the structures were placed back after having been removed, although this increase only reached the level

of a non-significant trend (χ^2 = 7.462, df = 3, n = 4, *P* = 0.053; Figure 2a). When the long wooden structures were present, however, the foxes showed significantly higher preferences for these enriched sectors compared to the rest of the enclosure (χ^2 = 8.1, df = 3, n = 4, *P* = 0.036, Figure 3a). The lowest median preference ratio occurred when the structures were removed from the enclosure (wREM; *post hoc* comparisons wREM < wLOC 1s = wLOC 2 = wLOC 1e, all *P* < 0.05).
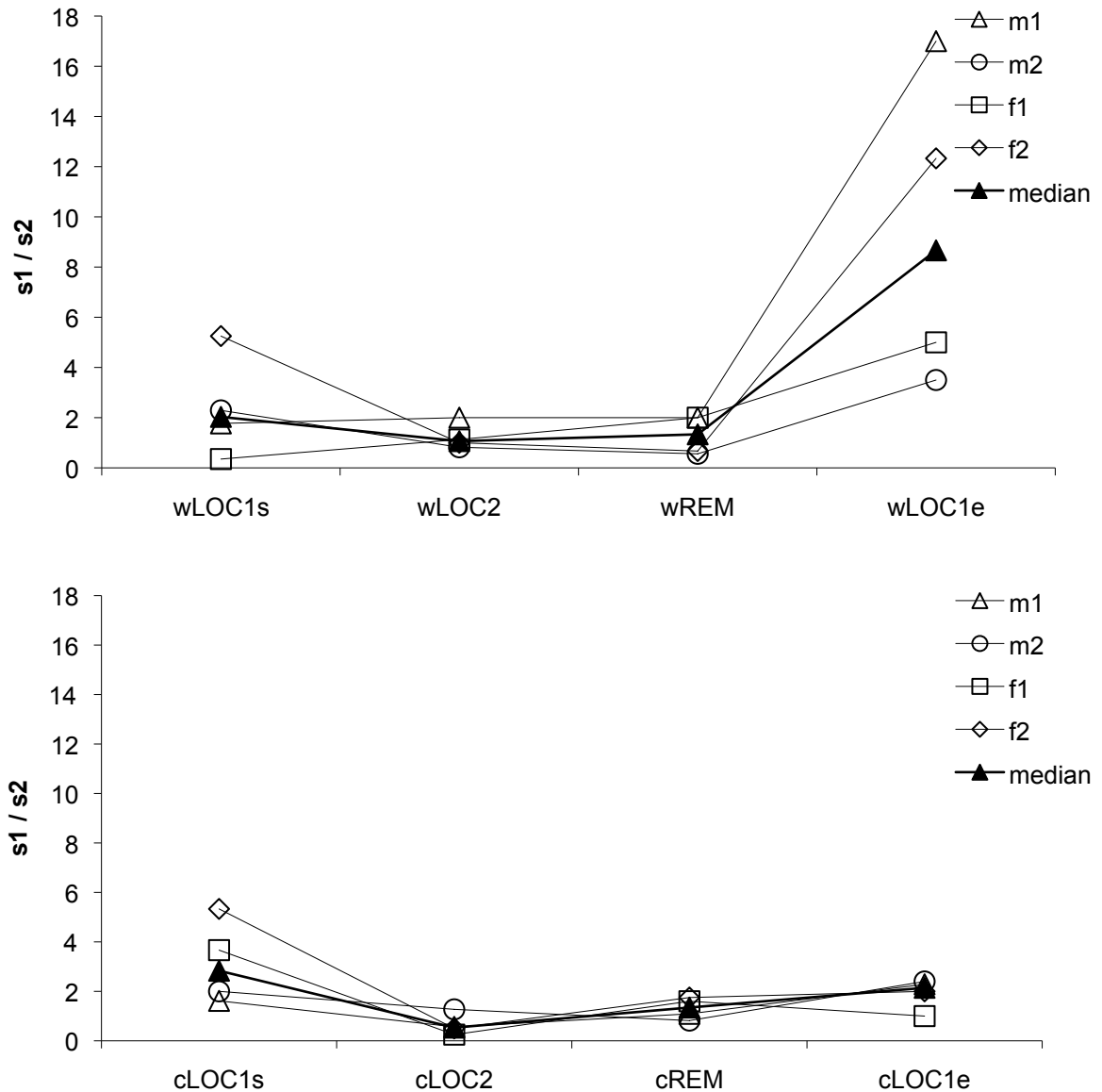

Figure 2: Individual use of experimental structures by four red foxes (two males, m1, m2, and two females, f1, f2) during four treatments (LOC 1s, LOC 2, REM, LOC 1e; for abbreviations of treatments see Material and methods), after enrichment with either long wooden structures (top) or cover structures (bottom). Ratios and overall medians are shown for s1/s2 (s1: sectors of the test enclosure containing structures during LOC 1s and LOC 1e; s2: sectors containing structures during LOC 2).

The use of sectors with cover structures differed significantly between treatments (χ^2 = 8.1, df = 3, n = 4, P = 0.036; Figure 2b), with the peak median ratio in the first treatment (cLOC 1s) and lowest median ratio when structures were dislocated (cLOC 2; *post hoc* comparisons cLOC 1s = cLOC 1e > cLOC 2 = cREM, all P < 0.05). The foxes did not differ significantly in their preferences for sectors with cover structures and after removal of such enrichment in comparison to the rest of the enclosure (χ^2 = 0.538, df = 3, n = 4, P = 0.946; Figure 3b).
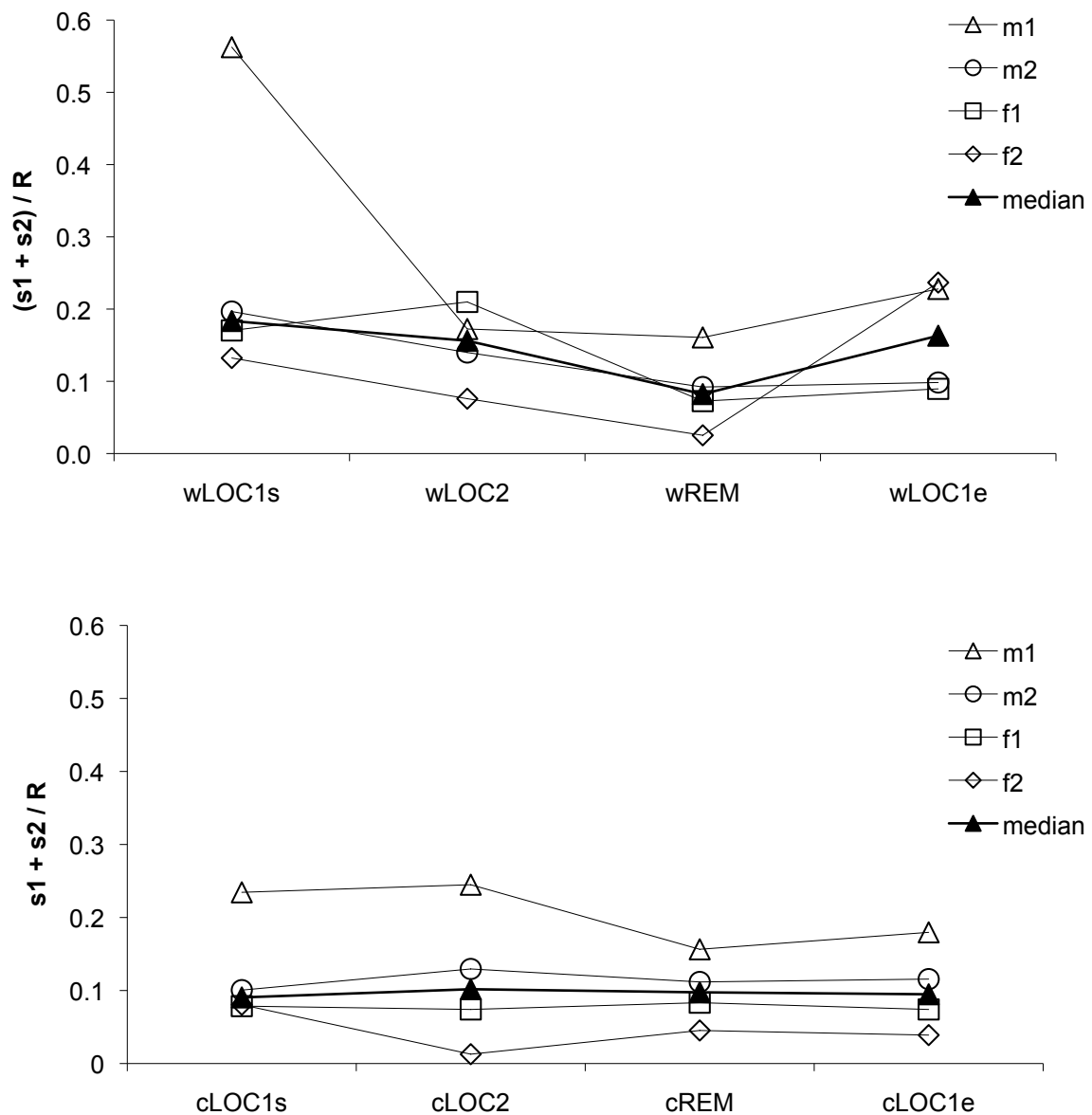

Figure 3: Individual preferences of sectors with experimental structures during four treatments (LOC 1s, LOC 2, REM, LOC 1e; for abbreviations of treatments see Material and methods), after enrichment with either long wooden structures (top) or cover structures (bottom). Ratios and overall medians are shown for (s1 + s2) / R (s1: sectors of the test enclosure containing structures during LOC 1s and LOC 1e; s2: sectors containing structures during LOC 2; R: rest of enclosure).

Effects of structural enrichment on activity in the test enclosure

In the experiment with the long wooden structures, overall activity of the foxes did not differ significantly between the four treatments (median activity ± se: wLOC 1s: 42.5 ± 0.68 %, wLOC 2: 38.6 ± 0.58 %, wREM: 42.3 ± 0.53 %, wLOC 1e: 49.2 ± 0.75 %; χ^2 = 8.1, df = 3, n = 4, *P* = 0.68).

In the experiment with cover structures, overall activity differed significantly between treatments (χ^2 = 8.4, df = 3, n = 4, *P* = 0.018), with similar median activity in treatments one (cLOC 1s: 55.2 ± 0.52 %) and two (cLOC 2: 56.4 ± 0.61 %) and decreasing median activity in treatment three (cREM: 52.1 ± 0.60 %) and four (cLOC 1e: 43.5 ± 0.33 %). *Post hoc* comparisons revealed that the foxes were least active in the treatment when the cover structures were placed back at location one after having been removed from the enclosure (cLOC 1e < cLOC 1s = cLOC 2 = cREM, all *P* < 0.05).

Use of structures in the large new enclosure

All four study individuals showed a significant overall preference for the eleven structures over comparable areas not providing structures in the large enclosure (Jacobs' preference indices: m1: t = 4.280, df = 10, *P* = 0.002; m2: t = 3.812, df = 10, *P* = 0.003; f1: t = 4.658, df = 10, *P* = 0.001; f2: t = 4.313, df = 10, *P* = 0.002). Such preferences were significantly higher during the first two weeks compared to the third week (Z = -2.578, *P* = 0.007, n = 11, Figure 4).
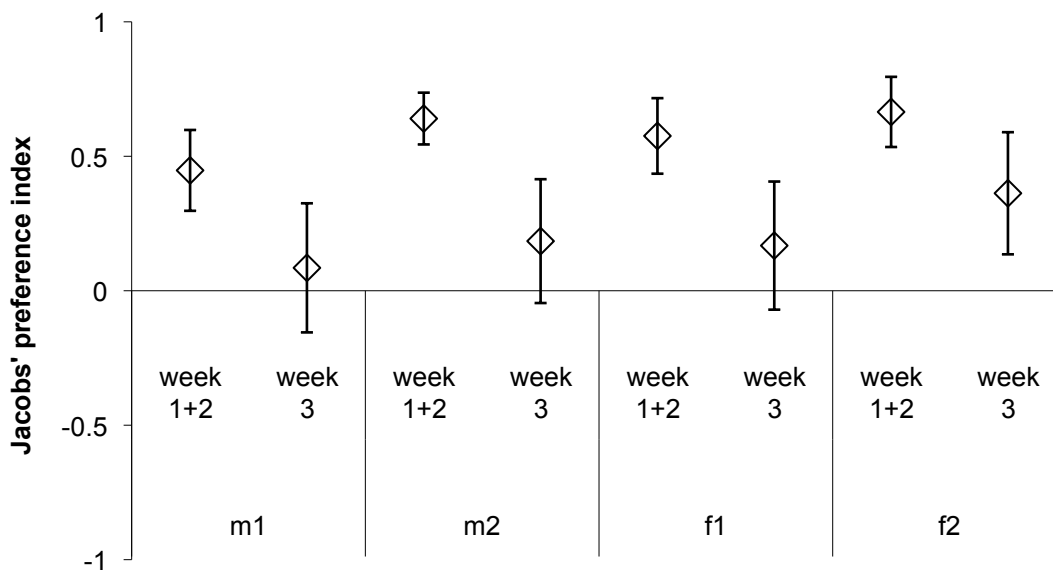

Figure 4: Jacobs' preference indices (see Methods for description) for the use of eleven areas with structures over comparably sized areas without structures in a large, natural-looking enclosure by four red foxes (two males, m1 and m2, and two females, f1 and f2). Positive values indicate preference, negative values avoidance. Individual means + SE for the period week 1+2 and week 3.

Activity in the new enclosure

Overall activity of the four foxes increased significantly over the three weeks observation period (χ^2 = 6.5, df = 2, n = 4, *P*= 0.042). *Post hoc* comparisons revealed a significant difference in activity between week 1 and 2, and between week 1 and week 3 (week 1 < week 2 = week 3, *P* < 0.05, Figure 5).
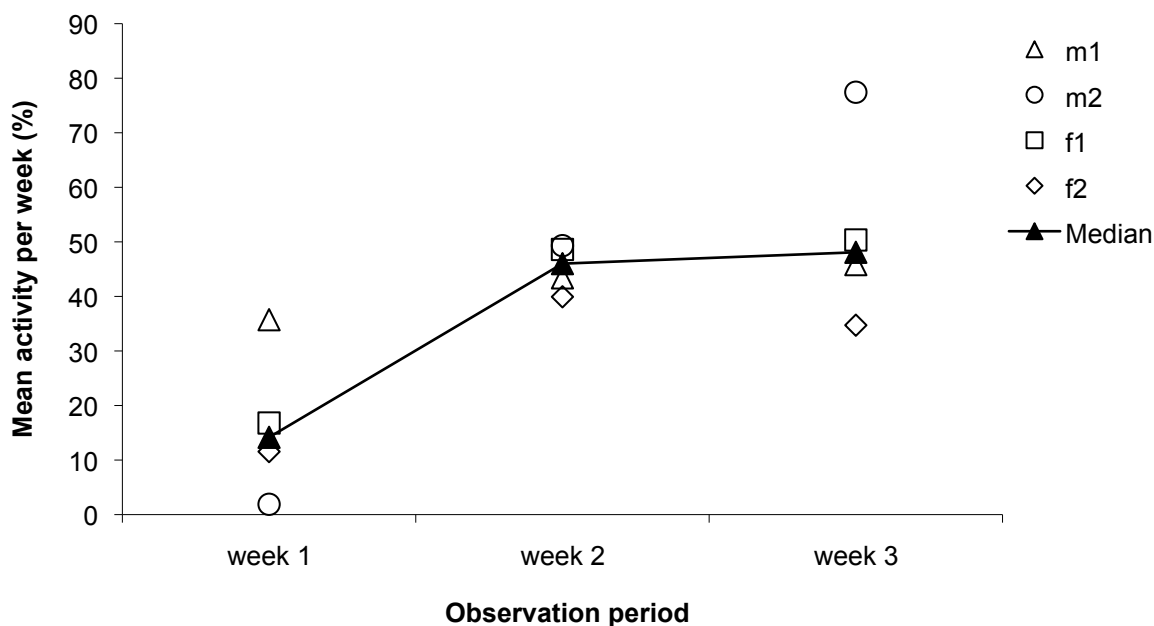

Figure 5: Percentage of activity (individual weekly mean and overall median) of four red foxes (two males, m1 and m2, and two females, f1 and f2) after transfer in a large natural-looking enclosure over a three-week study period.

Discussion

Influence of experimental structures on the behaviour

Introducing structural elements in the test enclosure affected the spatial behaviour of a group of two male and two female red foxes. The different features of the long wooden structures and the cover structures, respectively, had different effects on the foxes' spatial behaviour. The four subjects may have influenced each other in their behaviour. In family groups of red foxes, interactions between all members occur, although with different frequencies depending on dominant or subordinate status (Baker & Harris 2000). However, foxes range alone in their territory though they might follow the same routes (Macdonald 1988, Doncaster & Macdonald 1997), and are solitary foragers which do not share

food with conspecifics (Contesse *et al* 2004, Kistler *et al* 2009). Therefore, we treated the four individuals as statistically independent units with respect to the outcome measures taken.

The foxes generally preferred sectors containing long wooden structures over the rest of the enclosure, but showed only a non-significant tendency to preferentially use the relocated long wooden structures. The long wooden structures were concentrated in the flat part of the enclosure. If the foxes ranged here, they preferably used these long structures to move along. Thus, foxes do no arbitrarily range in the enclosure but orientate themselves along structures.

The cover structures, on the other hand, were distributed throughout the entire enclosure. The foxes shifted their use of sectors depending on the location of the cover structures, but did not prefer the respective sectors ahead of the rest of the enclosure. Cover structures are used for concealment when there is a threat (Weber & Meia 1996, Gloor 2002). The test enclosure contained a diversity of permanent natural structures, such as short hedges consisting of small fur trees and bushes, two dead trees, and several resting and hiding places above ground. Most of the structures could not be moved, either as being to large (such as the dead trees) or because they were planted, such as the small fur trees or bushes. It was almost certainly the case that permanent structures interfered with the experimental cover structures, such that the attractiveness of the experimental structures was relatively low. After completing data collection, the experimental structures were left in the enclosure, and over time trails were established through cover structures resembling trails found in the wild (C Kistler, personal observation 2007). This suggests that a longer period of habituation to the cover structures might have resulted in more intense usage.

Long structures are used to move along and also serve as barriers against humans and conspecifics (Blaney & Wells 2004, Aschwanden *et al* 2009). Foxes have a great fear of humans and tend to avoid them wherever possible (Lucherini *et al* 1995). Urban foxes would appear to have adapted to the presence of humans, but nevertheless show a preference for locations where human activity is low (Gloor 2002). Therefore retreat and hiding places should be offered in captivity (Carlstead 1991, Nimon & Broom 2001). In combination, long structures and cover structures may allow the foxes to safely explore their environment and forage for food. After introduction into the large, new enclosure, a strong preferences was shown for similar structures from the very beginning. These results support

the hypothesis that species living in cover-rich habitats benefit from enclosures with adequate structural enrichment.

It has been shown that housing conditions affect brain development and behaviour (Würbel 2001). The foxes had been raised as cubs in the test enclosure that had been richly structured in order to provide a stimulating environment and enable the development of normal behaviour. Feeding enrichment was provided which proved to enhance behavioural diversity and activity (Kistler *et al* 2009). The success of this environmental enrichment is reflected in the fact that none of the four individuals developed behavioural abnormalities, such as stereotypies, either in the test enclosure or in the new enclosure.

Effect of structural enrichment on activity

The foxes did not show enhanced activity in treatments with experimental structures present. However, activity level was high during both experiments. This could have been because the test enclosure was furnished, in addition to the experimental structures, with various other natural structures. Therefore, shelter and hiding places were always in close proximity when the foxes were moving throughout the enclosure. The reduced activity during the final treatment in the experiment on cover structures (cLOC 1e) was probably a direct result of road construction work carried out nearby. Nevertheless, the foxes selectively used the experimental cover structures during this period when they were ranging in the enclosure.

The newly built, natural-looking enclosure spanned 4'000m^2 and was larger than the test enclosure in which the foxes grew up by a factor of 13. Distances between structures were longer, and more open space was available. As expected, the foxes used the structures selectively and showed pronounced preferences for areas containing structures compared to corresponding structures-less areas. In the course of the three-week observation period, all four individuals showed increased use of open space and increased activity. In the new enclosure, individual activity levels after three weeks were comparable to individual activity levels in the previous test enclosure. In both enclosures, feedings were comparable. Since in the test enclosure and in the new enclosure, respectively, food was provided at several alternating sites every day, the foxes had to search for food and, therefore, finding food was time consuming. The increase in activity correlated with a less-pronounced preference for

areas containing structures. This suggests that in a new and therefore potentially threatening situation, structural enrichment assumes great importance. Furthermore, even when foxes were more familiar with their new environment, they still appeared to prefer areas containing structures, although the preference was less pronounced.

Increased use of cover structures and lower activity levels after the transfer of the foxes might reflect a novelty effect. However, also after three weeks the foxes mainly used one of the structures to rest. Only rarely did they rest in open space. This would suggest that structural enrichment was important not only in the new situation following the transfer, but also when the enclosure became more familiar to the foxes. Therefore, the high percentage of cover-structure usage appeared to reflect a preference for structures of the foxes and cannot be fully explained by a novelty effect. The interpretation of our results is also supported by other studies which attribute abnormal behaviours to the lack of secure hiding places (Carlstead 1991, Nimon & Broom 2001).

We did not measure any physiological correlate of stress. Nevertheless, we suggest that increased activity indicates improved well-being because structural enrichment enables animals to explore the enclosure and forage for food more safely. Red foxes are known to decrease activity when they are disturbed by human activity and shift their activity to more tranquil areas and those with dense cover (Cavallini & Lovari 1991, Gloor 2002, White *et al* 2006). Wild foxes are not only active during the night but also throughout the day, but mostly in areas where there is not much human activity (Zabel & Taggart 1989, Cavallini & Lovari 1991, Ricci *et al* 1998), and they tend to prefer to move in cover-rich habitats (Lucherini *et al* 1995). In the wild, foxes repeatedly rest during their active periods (Weber *et al* 1994, Doncaster & Macdonald 1997) and use different resting sites (Furrer 1999, Marks & Bloomfield 2006, White *et al* 2006). Hence, structural enrichment was placed such that the foxes were always able to retreat and hide or to move along a nearby structure while ranging. Our results indicate that a net-like arrangement of structural enrichment, including cover and long structures, is likely to be the most adequate furnishing to influence enclosure use.

Animal welfare implications

Previous studies have shown that stereotypies and other abnormal behaviours occur frequently in captive animals and can have a detrimental effect on welfare (for a review see Mason *et al* 2007).

Such behavioural disturbances arise when enclosures lack critical resources and stimuli that facilitate species-typical behaviour (Mason 1991). Mason *et al* (2007) regard environmental enrichment as the most adequate means to solve these problems. Adequate environmental enrichments can be identified and tested by using a naturalistic approach applied in this study. Providing captive animals with a richly structured environment and an adequate feeding enrichment (e.g. Kistler *et al* 2009) throughout their life may be crucial because housing conditions affect brain development and behaviour (Würbel 2001). The development of normal behaviour is most important for animals bred for reintroduction in conservation programmes (Rabin 2003), but also for the well-being of captive animals in general.

Conclusion

In captivity, structural enrichment has proven to be important for species that live in cover-rich habitats and suffer high risks of predation (Ödberg 1987, Buchwalder & Wechsler 1997, Wiedenmayer 1997, Würbel *et al* 1998). Therefore, to establish a species adequate enclosure, the natural environment the species has evolved in has to be taken into consideration. Providing red foxes with elements simulating natural structures, such as hedges or thickets had a profound effect upon their use of the enclosure. All individuals showed a preference for moving along long wooden structures and shifted their use of sectors depending on the location of cover structures. However, permanent structures in the enclosure which also provided cover and hiding places probably interfered with the use of experimental cover structures. Also in the new enclosure the foxes showed significant overall preference for structures and a preference for sectors containing structural enrichment compared to corresponding areas with no structural enrichment. In conclusion, both cover and long structures are important in enclosures that aim at providing captive foxes with a species adequate environment. In combination, they may meet the ecological and behavioural needs of an opportunistic carnivore.

ACKNOWLEDGEMENTS

REFERENCES

Adkins CA and Stott P 1998 Home ranges, movements and habitat associations of red foxes *Vulpes vulpes* in suburban Toronto, Ontario, Canada. Journal of Zoology 244: 335-346

Altmann J 1974 Observational study of behavior - sampling methods. Behaviour 49: 227-267

Aschwanden J, Gygax L, Wechsler B and Keil NM 2009 Loose housing of small goat groups: Influence of visual cover and elevated levels on feeding, resting and agonistic behaviour. Applied Animal Behaviour Science 119: 171-179

Baker PJ and Harris S 2000 Interaction rates between members of a group of Red Foxes (*Vulpes vulpes*). Mammal Review 30: 239-242

Blaney EC and Wells DL 2004 The influence of a camouflage net barrier on the behaviour, welfare and public perceptions of zoo-housed gorillas. Animal Welfare 13: 111-118

Broom DM 2007 Quality of life means welfare: how is it related to other concepts and assessed? Animal Welfare 16: 45-53

Buchwalder T and Wechsler B 1997 The effect of cover on the behaviour of Japanese quail (*Coturnix japonica*). Applied Animal Behaviour Science 54: 335-343

Carlstead K 1991 Husbandry of the Fennec fox *Fennecus zerda*: Environmental conditions influencing stereotypic behaviour. International Zoo Yearbook 30: 202-207

Cavallini P and Lovari S 1991 Environmental factors influencing the use of habitat in the red fox, *Vulpes vulpes*. Journal of Zoology 223: 323-339

Conover WJ 1980 Practical nonparametric statistics. 2nd Edition. Wiley: New York, USA

Contesse P, Hegglin D, Gloor S, Bontadina F and Deplazes P 2004 The diet of urban foxes (*Vulpes vulpes*) and the availabilty of anthropogenic food in the city of Zurich, Switzerland. Mammalian Biology 69: 81-95

Doncaster CP and Macdonald DW 1997 Activity patterns and interactions of red foxes (*Vulpes vulpes*) in Oxford city. Journal of Zoology 241: 73-87

Furrer CT 1999 Schlafplatzorte und Aufzuchtsplätze des Rotfuchses *Vulpes vulpes* in der Stadt Zürich
        - Angebot und Nutzung im urbanen Lebensraum. MS Thesis. University of Zurich, Switzerland

Gloor S 2002 The rise of urban foxes (*Vulpes vulpes*) in Switzerland and ecological and
        parasitological aspects of a fox population in the recently colonised city of Zurich. PhD Thesis.
        University of Zurich, Switzerland

Gusset M 2005 Faecal glucocorticoid level is not correlated with stereotypic pacing in two captive
        margays (Leopardus wiedii). Animal Welfare 14: 157-159

Hartmann M and Schiess M 1997 Ropes as climbing structures for clouded leopards (*Neofelis*
        nebulosa Griffith 1821). Proceedings of the 2nd International Conference on Environmental
        Enrichment 62-71. Copenhagen Zoo, Copenhagen, Denmark

Hartmann-Furter M 2000 A species-specific feeding technique for European wildcats (*Felis s.*
        sylvestris) in captivity. Säugetierkundliche Informationen 4: 567-575

Hediger H 1942 Wildtiere in Gefangenschaft. Eine Grundlage der Tiergartenbiologie. Benno Schwaber
        & Co. Verlag: Basel, Schweiz

Hughes BO and Duncan IJH 1988 The notion of ethological 'need', models of motivation and animal
        welfare. Animal Behaviour 36: 1696-1707

Hutchins M, Hancocks D and Crockett C 1984 Naturalistic solutions to the behavioral problems of
        captive animals. Der zoologische Garten N.F. 54: 28-42

Jacobs J 1974 Quantitative measurement of food selection. Oecologia 14: 413-417

Kistler C, Hegglin D, Würbel H and König B 2009 Feeding enrichment in an opportunistic carnivore:
        The red fox. Applied Animal Behaviour Science 116: 260–265

Lucherini M, Lovari S and Crema G 1995 Habitat use and ranging behaviour of the red fox (*Vulpes*
        vulpes) in a Mediterranean rural area: Is shelter availability a key factor? Journal of Zoology
        237: 577-591

Macdonald DW 1988 Running with the fox. Unwin Hyman: London

Mallapur A and Chellam R 2002 Environmental influences on stereotypy and the activity budget of
        Indian Leopards (*Panthera pardus*) in four zoos in Southern India. Zoo Biology 21: 585-595

Maple T and Perkins L 1996 Enclosure furnishing and structural environmental enrichment. In:
        Kleinmann D, Allen E, Thompson K and Lumpkin S (eds) Wild mammals in captivity: principles
        and techniques pp 212-222. University of Chicago Press: Chigago (IL), USA

Markowitz H 1982 Behavioural Enrichment in the Zoo. Van Nostrand Reinhold: New York, USA

Marks CA and Bloomfield TE 2006 Home-range size and selection of natal den and diurnal shelter sites by urban red foxes (*Vulpes vulpes*) in Melbourne. Wildlife Research 33: 339-347

Mason G, Clubb R, Latham N and Vickery S 2007 Why and how should we use environmental enrichment to tackle stereotypic behaviour? Applied Animal Behaviour Science 102: 163-188

Mason GJ 1991 Stereotypies: a critical review. Animal Behaviour 41: 1015-1037.

Mellen J, Stevens V and Markowitz H 1981 Environmental enrichments for servals, Indian elephants and Canadian otters. International Zoo Yearbook 21: 196-201

Nimon AJ and Broom DM 2001 The welfare of farmed foxes *Vulpes vulpes* and *Alopex lagopus* in relation to housing and management: A review. Animal Welfare 10: 223-248

Ödberg FO 1987 The Influence of cage size and environmental enrichment on the development of stereotypies in bank voles (*Clethrionomys glareolus*). Behavioural Processes 14: 155-173

Poole TB 1992 The nature and evolution of behavioural needs in mammals. Animal Welfare 1: 203-220

Rabin LA 2003 Maintainig behavioural diversity in captivity for conservation natural behaviour management. Animal Welfare 12:

Renner MJ and Lussier JP 2002 Environmental enrichment for the captive spectacled bear (*Tremarctos ornatus*). Pharmacology Biochemistry and Behavior 73: 279-283

Ricci S, Colombini I, Fallaci M, Scoccianti C and Chelazzi L 1998 Arthropods as bioindicators of the red fox foraging activity in a Mediterranean beach-dune system. Journal of Arid Environments 38: 335-348

Robinson MH 1998 Enriching the lives of zoo animals, and their welfare: where research can be fundamental. Animal Welfare 7: 151-175

Schetini de Azevedo CS, Cipreste CF and Young RJ 2007 Environmental enrichment: A GAP analysis. Applied Animal Behaviour Science 102: 329-343

Weber JM and Meia JS 1996 Habitat use by the red fox *Vulpes vulpes* in a mountainous area. Ethology Ecology & Evolution 8: 223-232

Weber JM, Meia JS and Aubry S 1994 Activity of foxes, *Vulpes vulpes*, in the Swiss Jura mountains. Zeitschrift für Säugetierkunde 59: 9-13

White JG, Gubiani R, Smallman N, Snell K and Morton A 2006 Home range, habitat selection and diet of foxes (*Vulpes vulpes*) in a semi-urban riparian environment. Wildlife Research 33: 175-180

Wiedenmayer C 1997 Causation of the ontogenetic development of stereotypic digging in gerbils. Animal Behaviour 53: 461-470

Würbel H 2001 Ideal homes? Housing effects on rodent brain and behaviour. Trends in Neurosciences 24: 207-211

Würbel H, Chapman R and Rutland C 1998 Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. Applied Animal Behaviour Science 60: 69-81

Zabel CJ and Taggart SJ 1989 Shift in red fox, *Vulpes vulpes*, mating system associated with El Niño in the Bering Sea. Animal Behaviour 38: 830-838

Zar JH 1999 Biostatistical analysis. 4th Edition. Prentice Hall: New Jersey, USA

CHATPTER 3

The effect of environmental complexity on behaviour of ornamental fish of the Cyprinidae family

To be submitted

CHATPTER 3

The effect of environmental complexity on behaviour of ornamental fish of the Cyprinidae family

Claudia Kistler[a], Daniel Hegglin[b], Hanno Würbel[c], Barbara König[a]

[a] Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland
[b] SWILD, Urban Ecology & Wildlife Research, Wuhrstrasse 12, Zürich, Switzerland
[c] Division of Animal Welfare and Ethology, Clinical Veterinary Sciences, University of Giessen, Germany

ABSTRACT

Ornamental fish are one of the most popular pets worldwide. However, information about their species adequate housing is hardly available, and the concept of environmental enrichment has been rarely applied to ornamental fish and to fish in general. Species diversity of ornamental fish is enormous, and therefore requirements for species-adequate housing may differ at least at the genus level. In our study, we chose six species of barbs and the zebrafish, which are commonly held in home aquaria, and the latter is also widely used as model species in laboratories. We conducted a choice experiment to test for the influence of environmental complexity on compartment preference and behaviour of the fish. 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 nine zones of similar size by defining three vertical layers and three horizontal areas (back vs. middle vs. front area). One group of chinese barbs, checker barbs, tiger barbs, fiveband barbs, ticto barbs, and black ruby barbs of eight to nine individuals each, and two groups of nine zebrafish each were observed on four consecutive days to assess compartment use, and on three consecutive days to assess behavioural diversity and use of zones within compartments. All groups of barb species, except tiger barbs, and both zebrafish groups showed a preference for the structured compartment. Over all species, behavioural diversity and space use did not differ between the empty and the structured compartment. However, there was an interesting variation in social behaviour between the species, probably due to different reproduction behaviours and mating tactics. Our results suggest, that barb species and zebrafish have a preference for a complex environment.

However, species-specific social behaviour may have had an influence on the fish' decision which compartment to choose. Studies conducted on a species and individual level could yield more detailed information about the behavioural and ecological needs of common ornamental fish species.

Keywords: Environmental enrichment, structural preference, animal welfare, ornamental fish

INTRODUCTION

Ornamental fish are popular pets and millions of freshwater and marine ornamental fish are traded worldwide (Chapman, 2000). There is also a growing trend to establish aquaria in public places for reasons of aesthetics (Soo and Todd, 2009). Whereas most of the fish kept in private aquaria are freshwater species, and many of these species are bred in captivity, mainly marine ornamental fish are captured in the wild because they are hard to raise in captivity (Tlusty, 2002). There is an enormous variety in ornamental fish (Riehl and Baensch, 1983). Despite the high numbers of animals and species concerned, scientific work about ecological and behavioural needs of ornamental fish is rare and most information about keeping ornamental fish is anecdotal. Fish, though, are animals with a sophisticated behaviour and welfare concerns increase due to growing knowledge about fish behaviour, brain anatomy and physiology (Huntingford et al., 2006). Fish welfare can be impaired when water quality (as pH or temperature), feeding, but also social partners or physical conditions are not adequate (Kolle and Hoffmann, 2001). Therefore, studies on environmental and behavioural enrichment in ornamental fish are needed to determine their species-specific needs.

Environmental enrichment is a concept that is widely used in laboratory and zoo animals, and to a lesser extent in pet animals, to improve the animals' welfare (Young, 2003). Environmental enrichment techniques refer to the natural habitats the animals are adapted to and the animals' natural behaviour, and aim to enable the animals to show their behavioural repertoire in captivity. In the aquarist literature, many ornamental freshwater species are described to live in rivers with vegetation, and therefore it is usually recommended to add plants to the aquarium. Nevertheless, ornamental fish are often held in a poor environment with no places to retreat, a problem that is also common in locations such as restaurants, hairdressers or lobbies where aquaria mainly serve as accessories or partitions (pers. observation). So far, only few studies have been conducted that deal with species-adequate housing in pet fish. For some species, ground substrate is regarded as an important structure that allows the fish to show natural behaviour (Galhardo et al., 2009; Mendonca et al., 2010). Environmental features such as the colour of aquaria backgrounds or available shelters may have an influence on behaviour and well-being (Serra et al., 1999; Barcellos et al., 2009). Another important issue is an adequate social environment, in particular group composition and group size (Etscheidt, 1995; Saxby et al., 2010). Often, mixed groups of the same genera are held together in the same aquarium, and although they might occur in the same habitat, their behavioural and ecological needs

might be different. Structural enrichment might also have an impact on reproductive behaviour and preference for spawning sites (Spence et al., 2007a). In the wild, females usually deposit eggs on (mostly silty) substrate among vegetation at the margins of water bodies (Spence et al., 2006).

In this study, we examined structure preferences of seven ornamental fish species belonging to the genera *Puntius* and *Danio* within the family of Cyprinidae. Barbs (*Puntius spp.*) are a group of fish that is quite diverse in size, shape and colour patterns what makes them popular ornamental fish (Kortmulder, 1981). They occur in the tropical and subtropical regions of Africa and predominantly Asia (Riehl and Baensch, 1983). For our study we chose the following barb species: Chinese barb (*Puntius semifasciolatus*), checker barb (*Puntius oligolepis*), tiger barb (*Puntius anchisporus*), fiveband barb (*Puntius pentazona*), ticto barb (*Puntius ticto*), black ruby barb (*Puntius nigrofasciatus*). All species are native to Southeast Asia, inhabit small, shallow and well vegetated waters where the water current is slow, and they live in shoals (Kortmulder et al., 1978; Riehl and Baensch, 1983). Furthermore, we chose the zebrafish (*Danio rerio*), also a common ornamental fish and a widely used animal model in research (Vascotto et al., 1997; Spence et al., 2008). 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).

The fish were exposed to an experimental situation where they had to actively choose between different compartments. Two semi-transparent plates of Plexiglas separated the compartments. One compartment was structured with plants and clay pots, and the other one was left empty. Between these two compartments there was a smaller compartment where food was offered. It was expected that all species would prefer the structured compartment to the empty compartment. Along with the more intense use of the structured compartment, we expected a higher behavioural diversity in this compartment. Furthermore, we expected that the fish would use the space more evenly in the structured compartment because structures can fulfil different behavioural functions such as providing cover or hiding opportunities against aggressive conspecifics or other fish species, and make space more accessible to the fish. In the empty compartment, we expected a preference for edges, the lower space containing a layer of sand, or the surface because these features would offer some cover or orientation, hence a less even use of space.

MATERIALS AND METHODS

Subjects and housing

The study was conducted in an indoor facility for animal housing. 9 chinese barbs (*Puntius semifasciolatus*), 8 checker barbs (*Puntius oligolepis*), 9 tiger barbs (*Puntius anchisporus*), 8 fiveband barbs (*Puntius pentazona*), 8 ticto barbs (*Puntius ticto*), 9 black ruby barbs (*Puntius nigrofasciatus*), and 2 x 9 zebrafish (*Danio rerio*) were obtained from a pet shop. All fish were adults. The groups were placed in eight different aquaria of slightly variable size (due to availability of aquaria; Table 1).

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. The backsides of the aquaria were shaded with black tissues on the exterior. Water temperature was 25 (± 1) °C and the light:dark cycle 12 h:12 h. 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.

Table 1. Indicated are sizes of the eight aquaria, group size of the six barbs species and zebrafish, and the position of the structural enrichment.

AQ	Size (lenght x width x height, cm)	Group size	Fish species	Structured compartment
1	130 x 50 x 50	9	Chinese Barb	right
2	100 x 50 x 50	8	Checker Barb	left
3	100 x 50 x 50	9	Tiger Barb	left
4	130 x 50 x 50	8	Fiveband Barb	right
5	130 x 50 x 50	8	Ticto Barb	left
6	120 x 40 x 40	9	Black Ruby Barb	right
7	160 x 40 x 50	9	Zebrafish	left
8	130 x 50 x 50	9	Zebrafish	right

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 and fiveband barbs, the walls were installed such that the holes were at the bottom of the wall, and with the other species the holes were at the top of the walls. Pilot studies had shown that the fish learnt 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 four 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 and Fig. 1). The middle compartment was smaller (distance between the walls: 30cm) and equipped with two internal filters, the heating element, and the automatic feeder on top of the tank. The fish were provided with food only in the middle compartment, thus motivating the fish to actively choose between the structured and the empty compartment after each feeding regime. 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. The fish were given the opportunity to habituate to the experimental setup during five weeks.
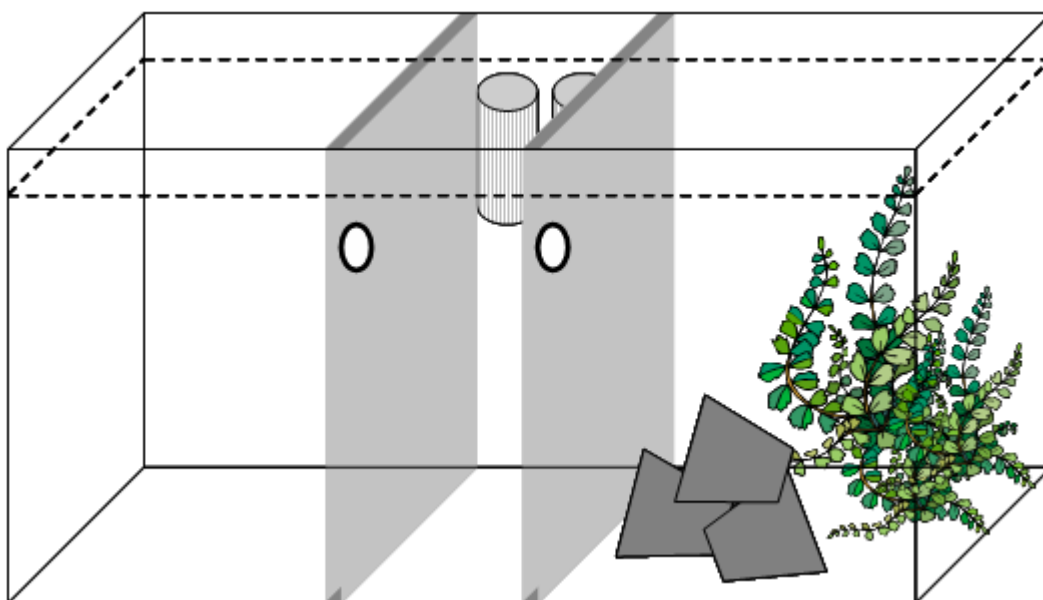

Figure 1. Design of the choice experiment. The aquarium was divided by two semitransparent plates into three compartments, the empty compartment (left), the structured compartment (right), and the food compartment (middle). The holes permitted the fish to change between the compartments. The structured compartment was supplied with plants and clay pots. The middle compartment contained two internal filters.

Data recording

To check for compartment use, behavioural data were collected on four consecutive days. Four times a day the position of all fish was recorded between 09.00 hours and 19.00 hours: two recordings 30 minutes after a feeding event in the morning (09.30 hours) and in the evening (18.30 hours), and two recordings 15 minutes after a feeding event at noon and in the early afternoon (14.00 hours). It was assumed that the choice between the left and the right compartment after feeding was independent of the previous position.

Behavioural data to assess behavioural diversity were collected on three consecutive days. Instantaneous observations at 5 min intervals were used to record the behaviour during twenty minutes. Four such recording bouts were distributed over the day between 10.00 hours and 17.30 hours. All behaviours were grouped into one of six functional categories (Table 2): exploration, foraging, resting, locomotion, social behaviour (socio-positive, socio-negative, mating), comfort behaviour plus waving. Waving describes a repeated movement: the fish swims to and for 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). In this study, it was not classified as stereotyped behaviour because this would require further observation for confirmation. 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. Along with the behaviour, the position of the fish in each compartment was recorded to evaluate space use. For this purpose, the aquarium was virtually divided vertically into three layers (lower layer, middle layer, upper layer) and horizontally in o three zones (back, middle, front), altogether into nine sectors of the same size: low front, low, middle, low back, middle front, middle middle, middle back, upper front, upper middle, 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.

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

Data analysis

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 two or more fish were observed in the structured and empty compartment together, 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 = (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 observation 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, being n the number of aquaria in the analysis).

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.

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

*H = - Σ (p$_i$ln * p$_i$)*

where *p$_i$* is the relative abundance of each functional category, calculated as the proportion of behavioural elements of a given functional category to the total number of behavioural elements of all functional categories: *n$_i$/N*. The index was calculated per aquarium. It increases with increasing

numbers of functional categories, and as the relative representation of each functional category becomes more even. Lower indices represent lower behavioural diversity. The Wilcoxon matched-pairs signed-rank test (Zar, 1999) was used to determine the differences between the empty and the structured compartment.

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

$SPI = M*[(n_b – n_a) / (F_a – F_b)] / 2* (N – M)$

where N is the total number of observations in all sectors, M the mean frequency of observations per sector (M/N), n_a the number of sectors with observations $> M$, n_b the number of sectors 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 sector; a value of 0 indicates maximum use, i.e. the fish would use all sectors 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. All significance tests were one-tailed.

RESULTS

Compartment preference

Mean percentage use of the structured and the empty compartment were 46% and 30% (chinese barb), 55% and 5% (checker barb), 19% and 73% (tiger barb), 61% and 13% (fiveband barb), 71% and 18% (ticto barb), 26% and 33% (black ruby barb), 40% and 28% (zebrafish 1), and 45% and 8% (zebrafish 2), respectively. Over all eight species, the fish showed a significant preference for the structured compartment (Jacobs' preference index: t = 1.928, df = 7, p = 0.048; Fig. 2).
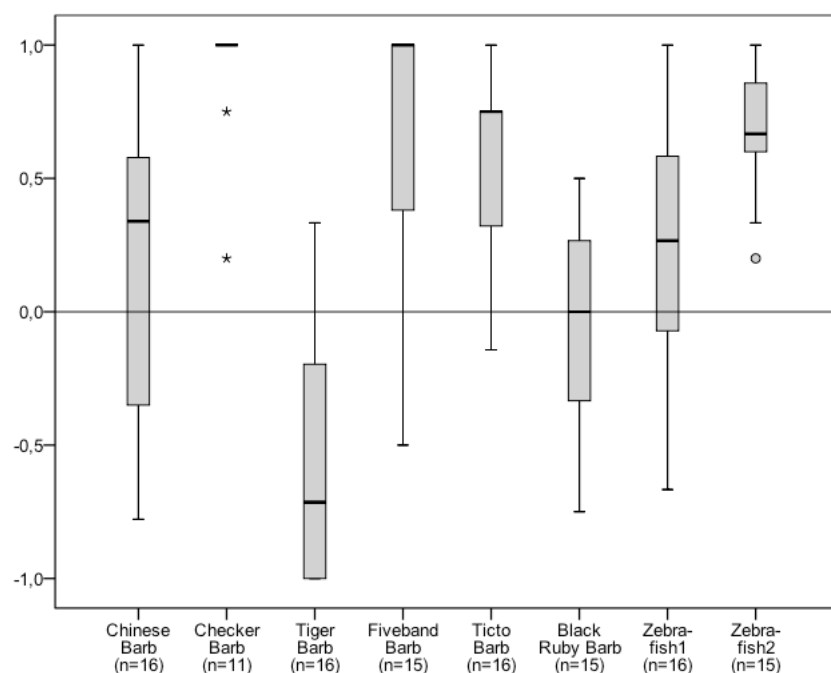
Figure 2. Jacobs' preference index for the use of the empty and the structured compartment for the six barbs species and the two zebrafish groups (n = number of independent sampling points). Positive and negative values indicate preference and avoidance, respectively; boxes indicate the 25±75th percentile range and contain the median line; bars represent the 10th and 90th percentile values; closed dots represent points outside these values.

In Table 3 mean number of fish per species in the structured and the empty compartment are

indicated.

Table 3. Values of mean shoal sizes, the Shannon diversity index and the Spread of participation index per species and per compartment.

AQ	Fish species	N (Samp-ling points)	Mean group size		Shannon diversity index		Spread of participation index	
			empty	*structured*	*empty*	*structured*	*empty*	*structured*
1	Chinese Barb	16	2.7 ± 0.5	4.1 ± 0.6	1.30	1.51	0.72	0.60
2	Checker Barb	16	0.4 ± 0.2	4.4 ± 0.8	0.69	1.30	0.90	0.64
3	Tiger Barb	16	6.1 ± 0.5	1.6 ± 0.4	1.29	1.17	0.66	0.37
4	Fiveband Barb	16	1.1 ± 0.4	4.9 ± 0.6	1.34	1.51	0.70	0.73
5	Ticto Barb	16	1.6 ± 0.3	6.3 ± 0.4	1.42	1.20	0.46	0.46
6	Black Ruby Barb	16	3.1 ± 0.5	2.5 ± 0.3	1.46	1.30	0.43	0.48
7	Zebrafish	16	2.1 ± 0.4	3.0 ± 0.3	0.94	0.98	0.24	0.26
8	Zebrafish	16	0.8 ± 0.8	4.3 ± 0.3	1.20	0.73	0.62	0.33

Behavioural diversity and sector use

Figure 3 shows the percentage of social behaviour (socio-positive, socio-negative, and mating behaviour) of the activity budget of all eight groups in the structured and in the empty compartment. Six of the eight groups showed socio-negative behaviours to higher percentages in the structured compartment than in the empty compartment.
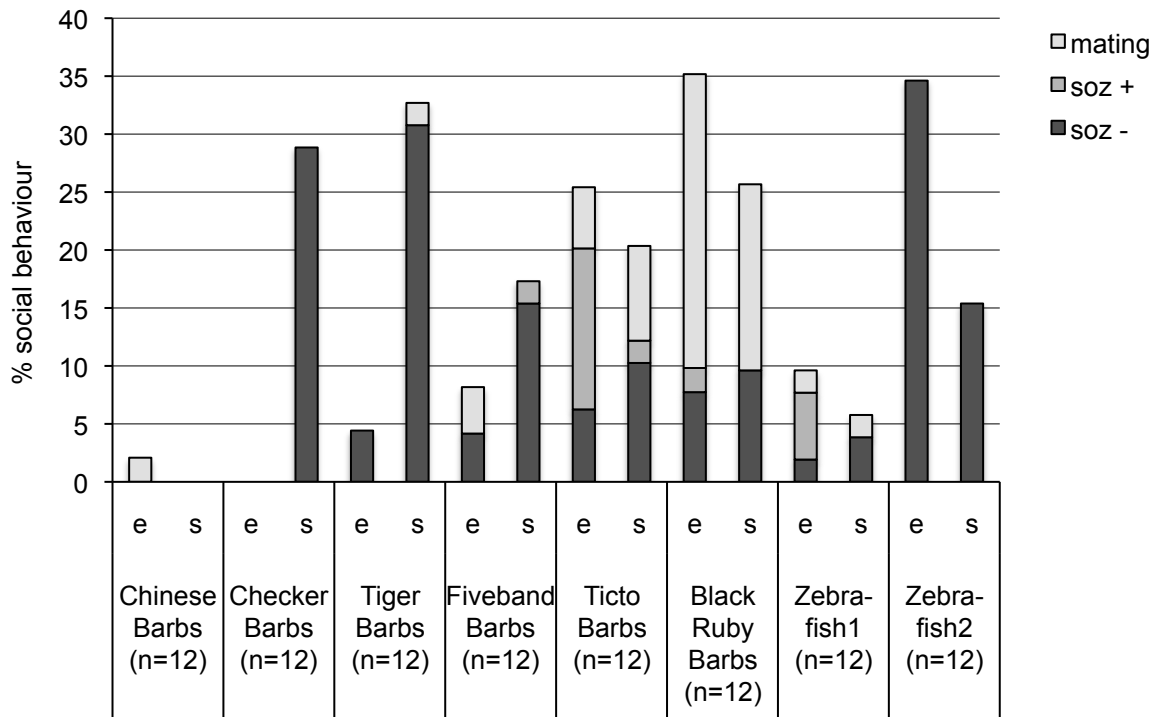

Figure 3. Percentage of the functional category social behaviour (mating = mating behaviour, soz+ = socio-positive behaviours, and soz- = socio-negative behaviours) on the activity budget of the six barbs species and the two zebrafish groups, (n = number of independent sampling points).

There was no significant difference in diversity of behaviour (Z = 0.00, p = 0.250, n = 8) and SPI (Z = -1.120, p = 0.132, n = 8) over all eight species between the empty and the structured compartment. Table 3 shows the Shannon diversity index and the spread of participation index per species.

DISCUSSION

Structure preference

Consistent with our expectations, a preference for the structured compartment over all eight groups was observed. The results confirm the hypothesis that Cyprinid species that naturally inhabit shallow waters with slow current and aquatic vegetation prefer a structured environment in the aquarium. Structural enrichment has proven to be important for the well-being of a wide range of animal species

held in captivity (e.g. in starlings Matheson et al., 2008; in hens Zeltner and Hirt, 2008; in foxes Kistler et al., 2010), including fish species such as the European minnow (*Phoxinus phoxinus*) and the South American catfish (*Rhamdia quelen*), which in the presence of shelter showed a significant better growth, and a reduced stress response, both in magnitude and duration, respectively (Wootton et al., 2006; Barcellos et al., 2009). Fish suffering high predation risks in their natural habitat might profit from cover also in captivity. In their study on black ruby barbs, Kortmulder et al. (1978) list several predators, amongst others freshwater garfish (*Xenetodon cancila*) and kingfisher (*Alcedo atthis*), species that might also predate on other barbs species. Also zebrafish suffer a high predation risk in their natural habitat (Engeszer et al., 2007). Although ornamental fish in private or public aquaria seldom encounter predators, they nevertheless might prefer a richly structured environment as structures serve different functions. Aquaria mimicking natural habitats offer stimulating environments and hiding places where the fish can retreat when disturbed by humans or other fish. For example, wild black ruby barbs were observed to hide near structures or below overhanging banks when they were disturbed (Schut et al., 1983).

However, interesting differences occurred between the species used in this study. Checker barbs showed the most pronounced preference for the structured compartment. Also fiveband barbs, ticto barbs, and one of the zebrafish groups clearly preferred the structured compartment. Chinese barbs and one of the zebrafish groups showed a less pronounced preference for the structured compartment, whereas black ruby barbs neither preferred nor avoided the structured compartment. Tiger barbs were the only species to avoid the structured compartment. These differences suggest that the species adapted differently to the experimental situation in which the compartments not only represented a structured or a barren environment, but probably also represented resources such as territories, and potential spawning or feeding sites. Although we did not distinguish between individuals, we observed that some individuals monopolised compartments and thus showed territoriality to some degree (see discussion below).

Behavioural diversity and sector use

Over all groups, behavioural diversity did not differ significantly between the structured and the empty compartment. The fish explored and foraged in both compartments, probably because some food (little pieces of flake food) that was not immediately caught diffused to the empty compartment.

Another explanation might be the variable social behaviour. Barbs differ in their reproductive behaviour, some are highly territorial during reproduction (e.g. tiger barbs), whereas others defend spawning sites, but do so not very aggressively (e.g. checker barbs, ticto barbs) (Kortmulder, 1981). According to Kortmulder (1981), black ruby barbs show a non-territorial aggregating type of reproductive behaviour. In our study, black ruby barbs engaged in mating behaviour such as following a partner or circling to a high degree in both compartments. This behaviour coincides with behavioural observations from their natural habitat where the first phase of courtship is performed in bare sections and mating (second phase) occurs between dense vegetation in shallow water (Kortmulder, 1981).

Male zebrafish adopt two mating tactics, territoriality by territorial males and active pursuit of females by non-territorial males. Territorial male zebrafish defend spawning sites, chase other males away and follow females only over short distances (Spence et al., 2007a; Hutter et al., 2010), behaviours that they also showed in our study. Moreover, zebrafish establish dominance hierarchies during mating (Spence et al., 2008) and foraging when dominant individuals try to monopolise food resources (Basquill and Grant, 1998). Also male tiger barbs are described to establish rank orders, to be territorial and to defend spawning sites (Kortmulder, 1981; Riehl and Baensch, 1983). Saxby et al. (2010) observed in tiger barbs increased shoaling behaviour with increasing group size (five and eight individuals, respectively), but no decrease in aggressive behaviour. In our study, socio-negative behaviours were frequent in all groups except for Chinese barbs, probably reflecting social dominance and monopolisation of resources. In tiger barbs, probably social dominance interfered with the preference for structures to a degree that the majority of the group avoided the structured compartment.

Overall, differences in social behaviour might have influenced the results of our study. Interestingly most of the groups showed more socio-negative behaviour in the structured compartment than in the empty compartment. Aggressive behaviour is part of the behavioural repertoire, and in particular part of territorial and social behaviour. Despite the higher amount of aggression in the structured compartment, the fish preferred to stay most of the time in this environment. This suggests that structures fulfil important functions, and moreover allow the fish to escape aggressions from conspecifics. The fact that in most barb species and in zebrafish group sizes were smaller in the empty than in the structured compartment supports the hypothesis that a structured environment

might allow fish to live in larger densities without being exposed to too high intraspecific aggression. Studies including observations on an individual basis could provide more detailed information about dominance hierarchies and social interactions.

Over all eight groups, the fish did not use the sectors more evenly in the structured compartment than in the empty compartment, in fact each species used both compartments in a similar way, except tiger barbs. Tiger barbs used the back sectors in the structured compartment more than in the empty compartment, where they mainly used the front sectors. On the one hand structures can serve as optical barriers, but on the other hand can cause territorial behaviour (Williams et al., 2009). Subordinate individuals probably avoided aggressive behaviour of dominant individuals by hiding in the structures, but also by switching to the empty compartment.

Altogether, the barb species used the front sectors and the lower sectors to a high degree. The backsides were shaded with black tissue, and the sides of the aquaria were protected by adjacent aquaria such that outside disturbances were kept to a minimum. Therefore, we cannot exclude that the increased use of the front sector reflected a reaction to outside events, although the observer did not move during data collection. Moreover, the holes allowing to swim between the compartments were situated in the front sector. The intense use of the lower sectors probably reflects the feeding habits. Desilva and Kortmulder (1976) analysed the diet of black ruby barbs and found that these barbs were predominantly herbivorous, nipping algae from stones and logs, and in addition to animal matter also sand and detritus was found to a significant amount. It can be assumed that the diet of the other barb species used in this study is similar as they occur in the same habitats. In our study, foraging behaviours such as dabbling (searching for food in the sand), rasping algae from the glass pane and picking plants were frequently observed. Wild zebrafish are omnivorous and feed in the water column, from the substratum, but also on or near the water surface (Spence et al., 2007b). This might explain their more even use of the sectors.

In contrast to welfare studies on zoo or farm animals, only few studies have investigated preferences in ornamental fish (Huntingford et al., 2006). In fish welfare, preference tests can be an adequate method for examining species-adequate housing and better welfare as physiological measures are difficult to obtain; normally the fish have to be killed for the analysis of stress hormones and non-

invasive procedures are still not well established and difficult to interpret (Scott and Ellis, 2007). Preference tests can be used as tool to ask what animals want and therefore improve their well-being (Dawkins, 2003).

In conclusion, all species observed in this study except tiger barbs showed a preference for the structured compartment, though to different degrees. Structures can serve as cover or food resources, but also as potential spawning sites, and can create a stimulating environment. In our study on six barbs species and zebrafish, social behaviour and the tendency to occupy territories probably interfered with the preference for a structured environment to some degree. It is assumed that the experimental design facilitated the establishment of territories in some of the species. Therefore, more detailed studies on a species and individual level are needed to verify the behavioural and ecological needs of ornamental fish species.

REFERENCES

Barcellos, L.J.G., Kreutz, L.C., Quevedo, R.M., da Rosa, J.G.S., Koakoski, G., Centenaro, L., Pottker, E., 2009. Influence of color background and shelter availability on jundia (Rhamdia quelen) stress response. Aquaculture 288, 51-56.

Basquill, S.P., Grant, J.W.A., 1998. An increase in habitat complexity reduces aggression and monopolization of food by zebra fish (Danio rerio). Can. J. Zool. 76, 770-772.

Carfagnini, A.G., Rodd, F.H., Jeffers, K.B., Bruce, A.E.E., 2009. The effects of habitat complexity on aggression and fecundity in zebrafish (Danio rerio). Environ. Biol. Fish. 86, 403-409.

Casamitjana, J., 2004. Aquatic Zoos. A Critical Study of UK Public Aquaria in the Year 2004. The Captive Animals' Protection Society.

Chapman, F.A., 2000. Ornamental fish culture, freshwater, In: Stickney, R.R.E. (Ed.), Encyclopedia of Aquaculture, John Wiley and Sons, Inc., pp. 602-610.

Dawkins, M.S., 2003. Behaviour as a tool in the assessment of animal welfare. Zoology 106, 383-387.

Desilva, S.S., Kortmulder, K., 1976. Some Aspects of Biology of 3 Species of Puntius-(= Barbus)- (Pisces, Cyprinidae), Endemic to Sri-Lanka. Neth. J. Zool. 27, 182-194.

Dickens, M., 1955. A Statistical Formula to Quantify the "Spread-of-Participation" in Group Discussion. Speech Monographs 22, 28-30.

Engeszer, R.E., Patterson, L.B., Rao, A.A., Parichy, D.M., 2007. Zebrafish in the Wild: A Review of Natural History and New Notes from the Field. Zebrafish 4, 21-40.

Etscheidt, J., 1995. Critical Aspects of the Welfare of Fresh-Water Fish in Pet Shops. Tierarztliche Umschau 50, 196-199.

Galhardo, L., Almeida, O., Oliveira, R.F., 2009. Preference for the presence of substrate in male cichlid fish: Effects of social dominance and context. Appl. Anim. Behav. Sci. 120, 224-230.

Huntingford, F.A., Adams, C., Braithewaite, V.A., Kadri, S., Pottinger, G., Sandoe, P., Turnbull, J.F., 2006. Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Hutter, S., Penn, D.J., Magee, S., Zala, S.M., 2010. Reproductive behaviour of wild zebrafish (Danio rerio) in large tanks. Behaviour 147, 641-660.

Jacobs, J., 1974. Quantitative measurement of food selection. Oecologia 14, 413-417.

Kistler, C., Hegglin, D., Würbel, H., König, B., 2010. Structural enrichment and enclosure use in an opportunistic carnivore: the red fox. Anim. Welfare 19, 391-400.

Kolle, P., Hoffmann, R., 2001. Mistakes in general husbandry of ornamental fish. Deutsche Tierarztliche Wochenschrift 108, 121-123.

Kortmulder, K., 1981. Etho-Ecology of 17 Barbus Species (Pisces, Cyprinidae). Neth. J. Zool. 32, 144-168.

Kortmulder, K., Feldbrugge, E.J., Desilva, S.S., 1978. Combined field-study of *Barbus (=Puntius) nigrofasciatus* Gunther (Pisces, Cyprinidae) and water chemistry of its habitat in Sri Lanka Neth. J. Zool. 28, 111-&.

Matheson, S.M., Asher, L., Bateson, M., 2008. Larger, enriched cages are associated with 'optimistic' response biases in captive European starlings (Sturnus vulgaris). Appl. Anim. Behav. Sci. 109, 374-383.

McClure, M.M., McIntyre, P.B., McCune, A.R., 2006. Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish Danio rerio. J. Fish Biol. 69, 553-570.

Mendonca, F.Z., Volpato, G.L., Costa-Ferreira, R.S., Goncalves-de-Freitas, E., 2010. Substratum choice for nesting in male Nile tilapia Oreochromis niloticus. J. Fish Biol. 77, 1439-1445.

Riehl, R., Baensch, H.A., 1983. Aquarienatlas. Mergus Verlag für Natur- und Tierheimkunde Baensch, Melle, Germany.

Saxby, A., Adams, L., Snellgrove, D., Wilson, R.W., Sloman, K.A., 2010. The effect of group size on the behaviour and welfare of four fish species commonly kept in home aquaria. Appl. Anim. Behav. Sci. 125, 195-205.

Schut, J., Desilva, S.S., Kortmulder, K., 1983. Habitat, associations and competition of 8 Barbus (=Puntius) species (Pisces, Cyprinidae) indigenous to Sri-Lanka Neth. J. Zool. 34, 159-181.

Scott, A.P., Ellis, T., 2007. Measurement of fish steroids in water - a review. General and Comparative Endocrinology 153, 392-400.

Serra, E.L., Medalha, C.C., Mattioli, R., 1999. Natural preference of zebrafish (Danio rerio) for a dark environment. Brazilian Journal of Medical and Biological Research 32, 1551-1553.

Shannon, C.E., Weaver, W., 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

Soo, P., Todd, P.A., 2009. Fish in Aquariums for Aesthetically Enhancing Public Spaces (AAEPS): An Incipient Welfare Issue? J. Appl. Anim. Welf. Sci. 12, 263-272.

Spence, R., Ashton, R., Smith, C., 2007a. Oviposition decisions are mediated by spawning site quality in wild and domesticated zebrafish, Danio rerio. Behaviour 144, 953-966.

Spence, R., Fatema, M.K., Ellis, S., Ahmed, Z.F., Smith, C., 2007b. Diet, growth and recruitment of wild zebrafish in Bangladesh. J. Fish Biol. 71, 304-309.

Spence, R., Fatema, M.K., Reichard, M., Huq, K.A., Wahab, M.A., Ahmed, Z.F., Smith, C., 2006. The distribution and habitat preferences of the zebrafish in Bangladesh. J. Fish Biol. 69, 1435-1448.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, Danio rerio. Biol. Rev. 83, 13-34.

Tlusty, M., 2002. The benefits and risks of aquacultural production for the aquarium trade. Aquaculture 205, 203-219.

Vascotto, S.G., Beckham, Y., Kelly, G.M., 1997. The zebrafish's swim to fame as an experimental model in biology. Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire 75, 479-485.

Williams, T.D., Readman, G.D., Owen, S.F., 2009. Key issues concerning environmental enrichment for laboratory-held fish species. Lab. Anim. 43, 107-120.

Wootton, R.J., Handisyde, N., Rowe, C., 2006. The effect of cover on food consumption and growth in two freshwater fish species used in experimental studies. Anim. Welfare 15, 309-313.

Young, R.J., 2003. Environmental Enrichment for Captive Animals. Blackwell Publishing Professional,

Ames, Iowa, USA.

Zar, J.H., 1999. Biostatistical analysis. Prentice Hall, New Jersey, USA.

Zeltner, E., Hirt, H., 2008. Factors involved in the improvement of the use of hen runs. Appl. Anim.

Behav. Sci. 114, 395-408.

CHAPTER 4

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

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

Claudia Kistler[a], Daniel Hegglin[b], Hanno Würbel[c], Barbara König[a]

[a] Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland

[b] SWILD, Urban Ecology & Wildlife Research, Wuhrstrasse 12, Zürich, Switzerland

[c] Division of Animal Welfare and Ethology, Clinical Veterinary Sciences, University of Giessen, Giessen, Germany

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.

Keywords: Environmental enrichment, structural preference, animal welfare, ornamental fish, laboratory fish, zebrafish, checker barb

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 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.

MATERIALS AND METHODS

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 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 2cm, 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.

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.
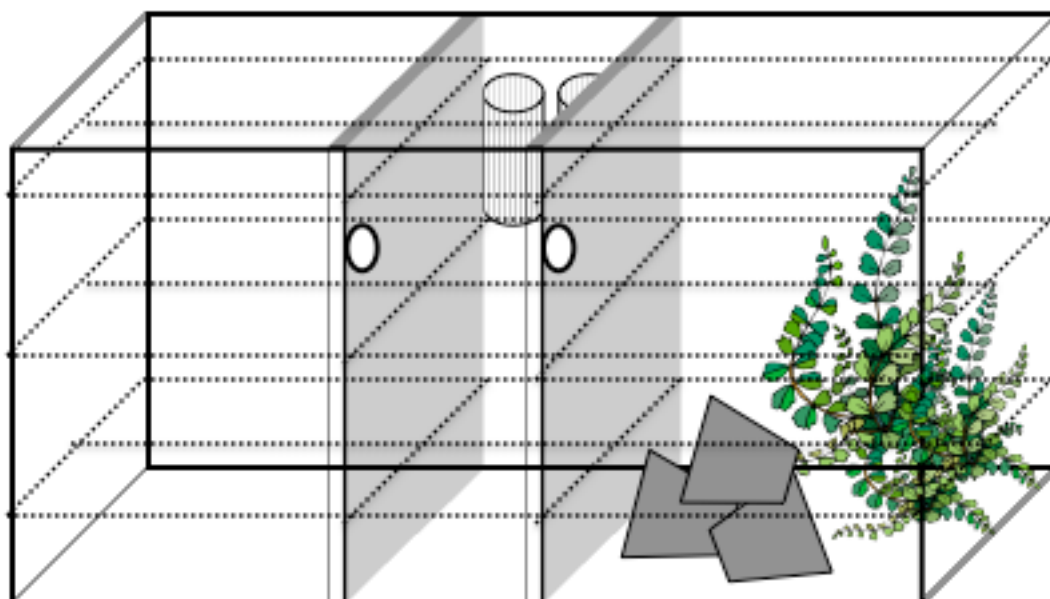
Figure 1. Design of the choice experiment. The aquarium was divided by two semitransparent plates into three compartments, the empty compartment (here left), the structured compartment (right), and the food compartment (middle). The holes permitted the fish to change between the compartments. The structured 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.

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 x width x height, cm)	Structured compartment	# Zebrafish	Zebrafish strains	# Checker Barbs
1	130 x 50 x 50	right	8	Tü + WIK	8
2	100 x 50 x 50	left	6	Albino	7
3	100 x 50 x 50	left	8	Tü + WIK	7
4	130 x 50 x 50	right	8	Tü + WIK	8
5	160 x 41 x 50	left	9	AB	7
6	130 x 50 x 50	right	8	Tü + WIK	7
7	100 x 50 x 50	left	9	pet shop	8

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 x 40 x 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 minutes after a feeding event in the morning (10.30 hours) and in the evening (16.30 hours), and two recordings 15 minutes 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 ten minutes 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, 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.

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

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.

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

$J = (r-p) / [(r+p) - 2rp]$

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 x 10min), 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

$SPI = M*[(n_b - n_a) / (F_a - F_b)] / 2* (N - M)$

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.

RESULTS

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 minutes 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 empty compartment), and 1.18 (± 0.09; middle to the structured compartment).
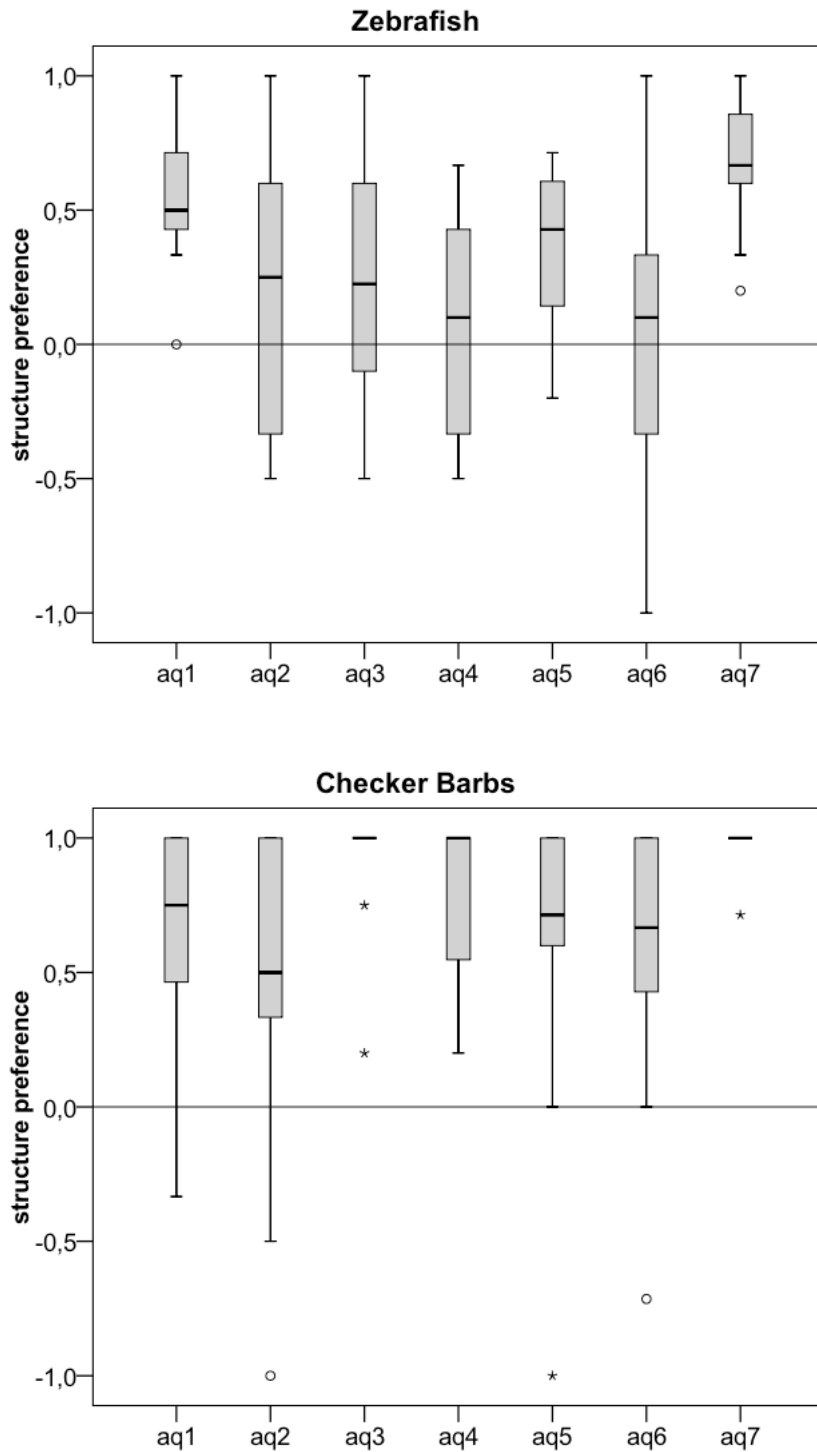
Figure 2. Jacobs' preference index for the use of the empty and the structured compartment in seven aquaria for (a) zebrafish and (b) checker barbs. Positive and negative values indicate preference and avoidance, respectively; boxes indicate the 25±75th percentile range and contain the median line; bars represent the 10th and 90th percentile values; open dots represent points outside these values.

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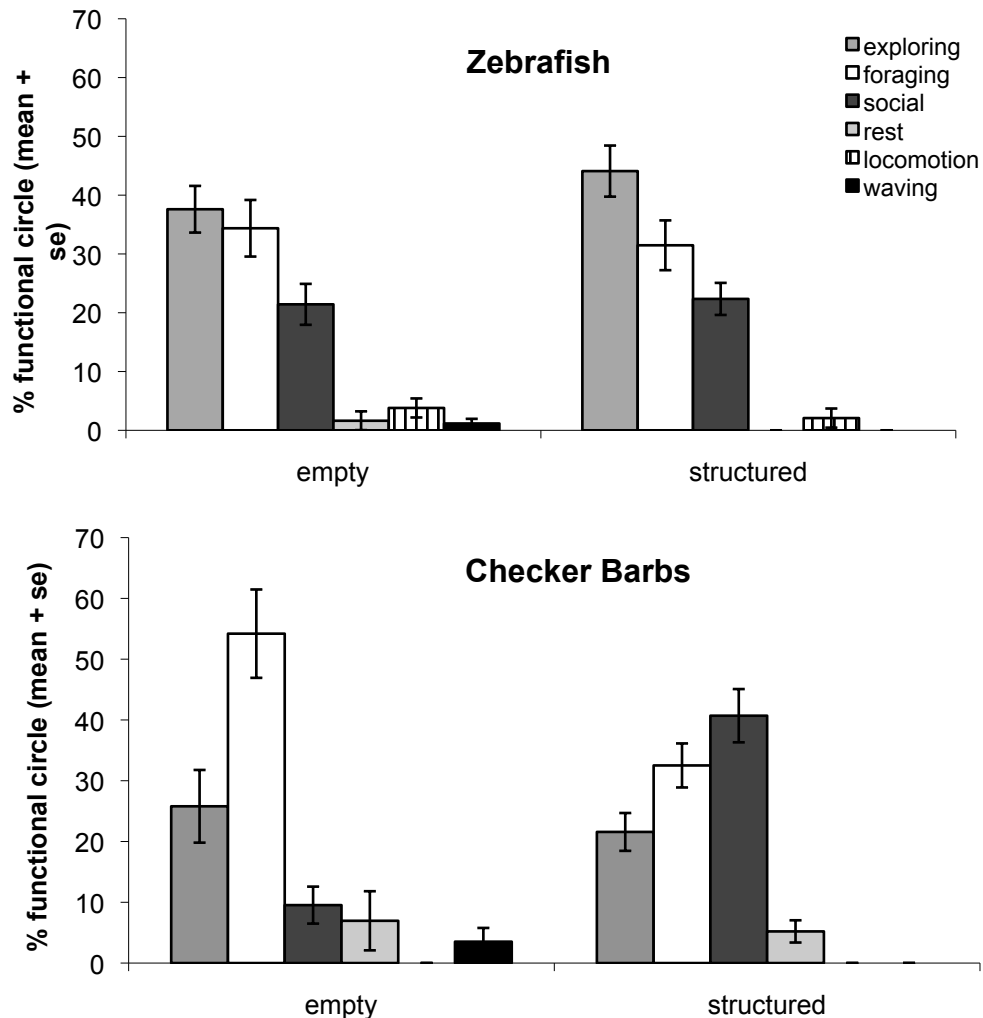
Figure 3. Activity budget (overall mean + SE) for (a) zebrafish (n = 6 aquaria) and (b) checker barbs (n = 6).

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; Fib 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).

Zebrafish
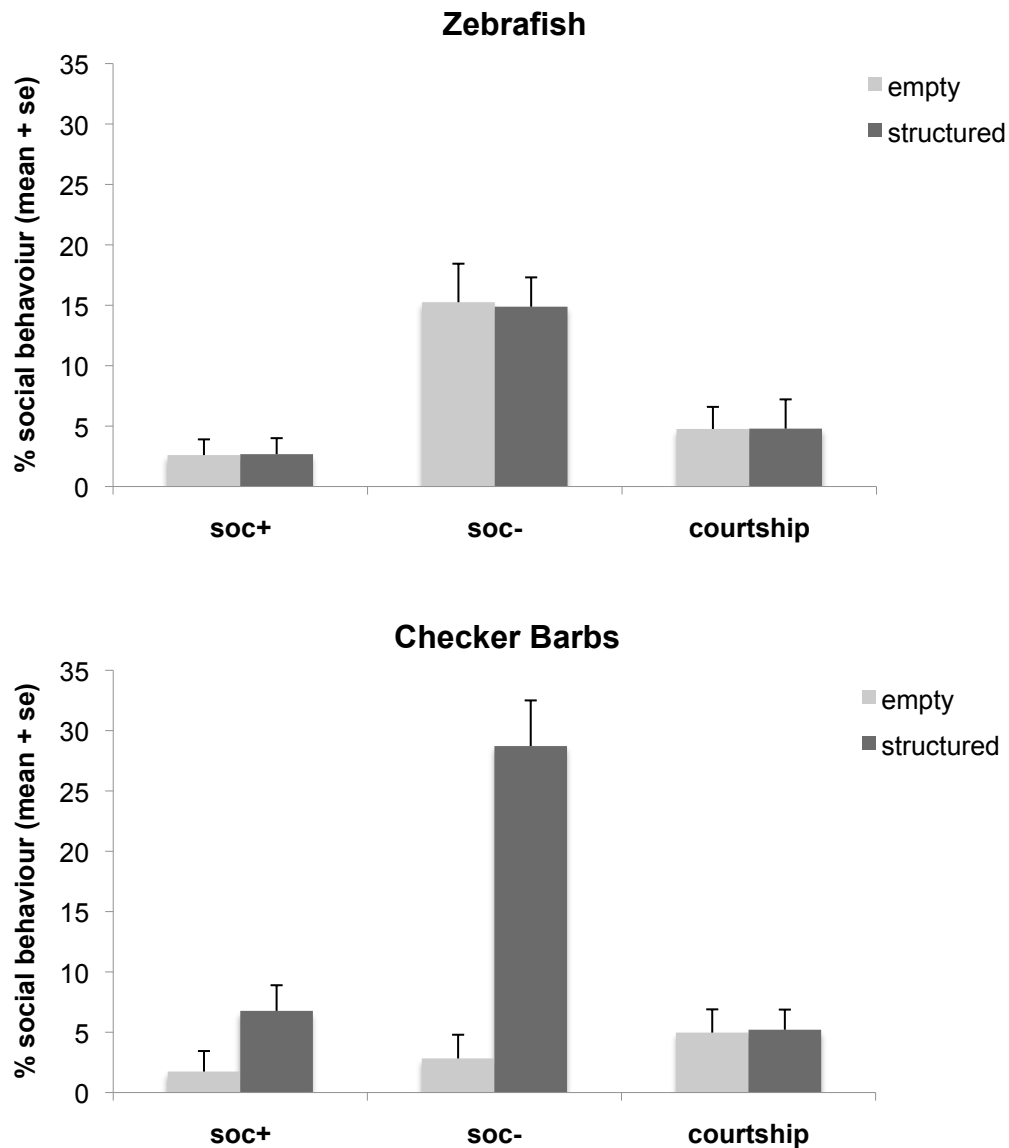
Checker Barbs

Figure 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).

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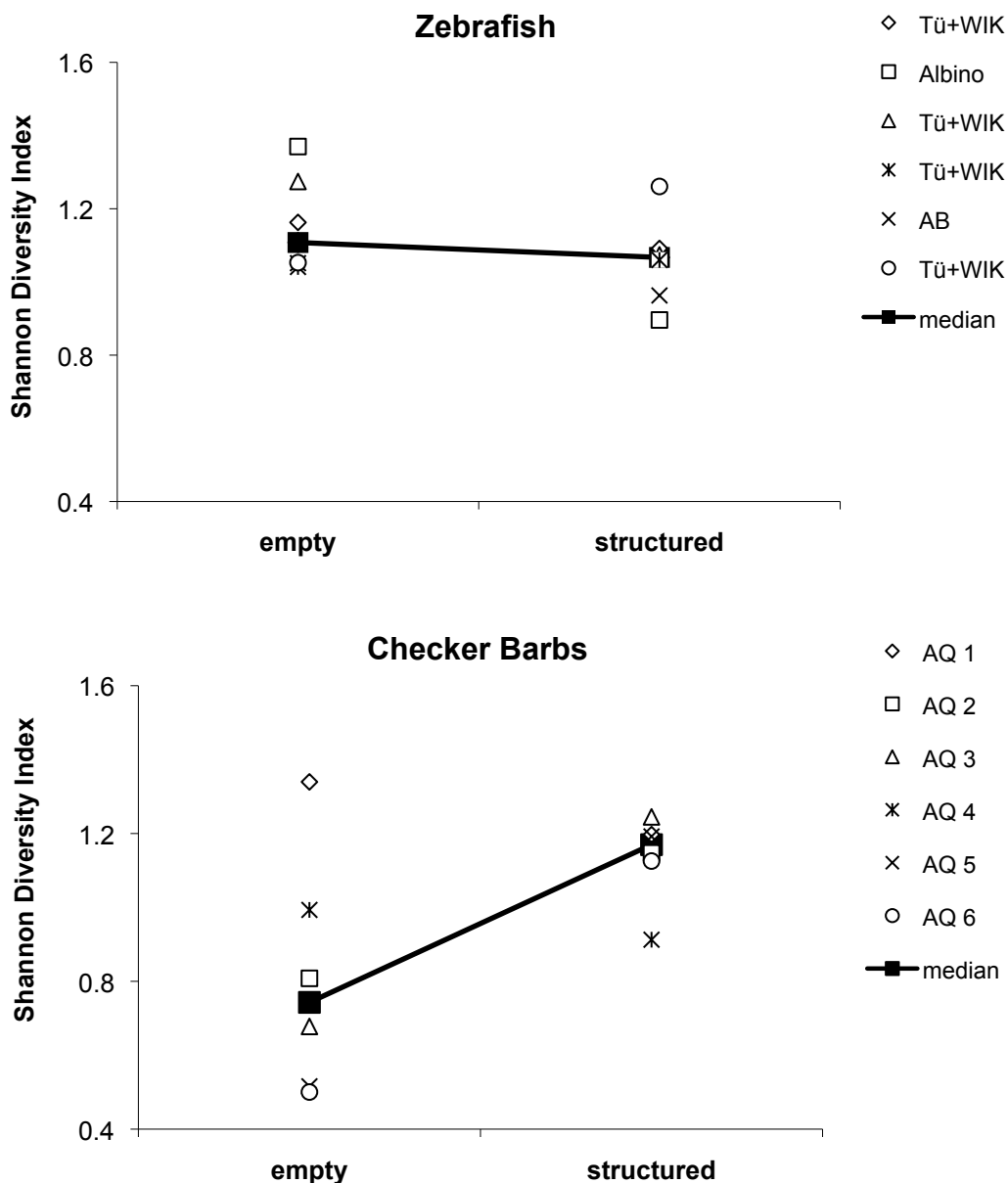
Figure 5. Behavioural diversity in the empty and the structured compartment (plant and pots) for (a) zebrafish and (b) checker barbs. Values of Shannon diversity index of six aquaria and overall median are shown. Lower index values represent lower behavioural diversity, and higher values represent higher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

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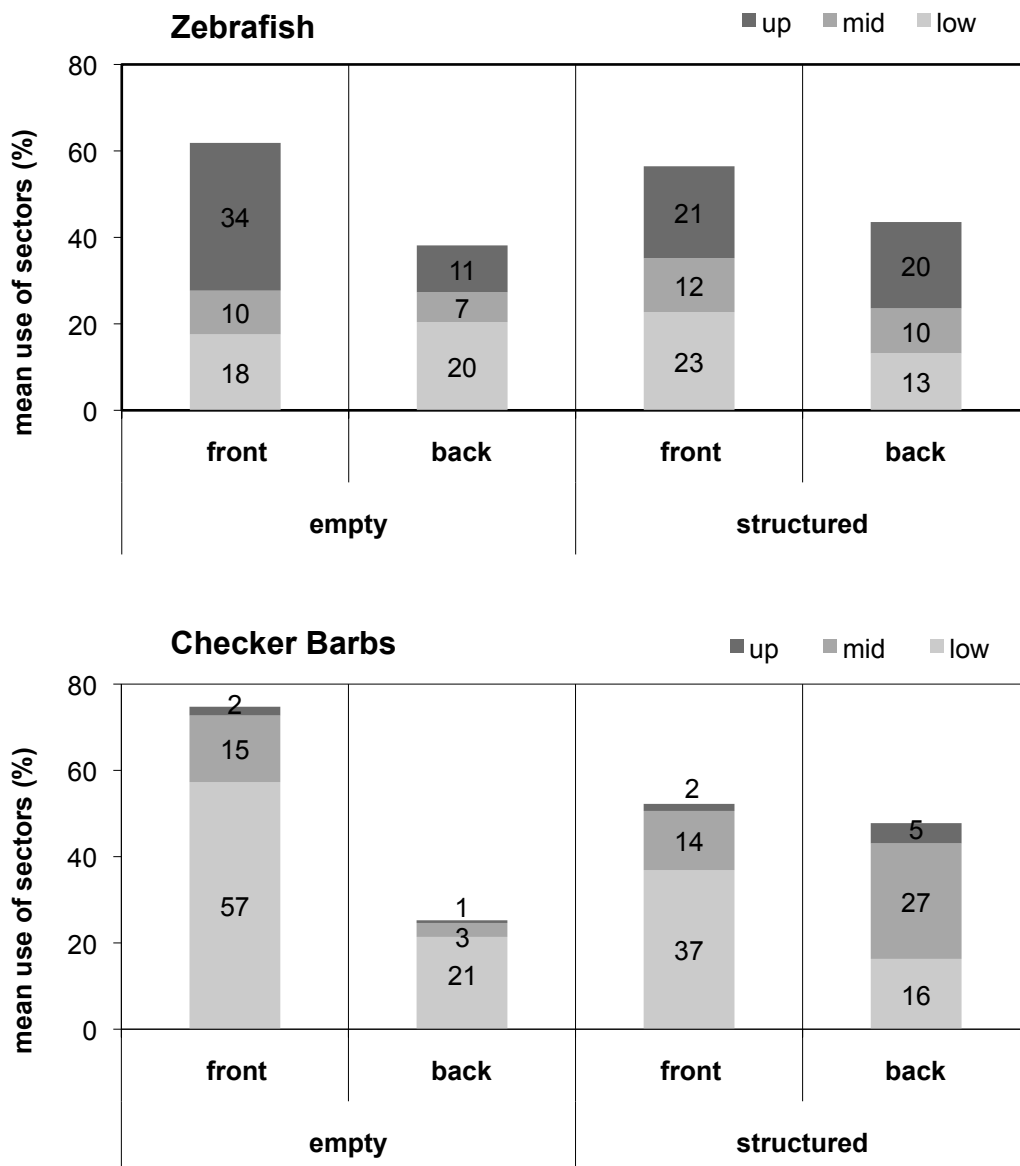
Figure 6. Mean use of sectors in the empty and the structured compartment for (a) zebrafish (n = 6) and (b) checker barbs (n = 6). The six sectors are: upper front, upper back, middle front, middle back, lower front, and lower back.

DISCUSSION

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 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.

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; Spence et al., 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.

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.

ACKNOWLEDGEMENTS

REFERENCES

Ashley, P.J., 2007. Fish welfare: Current issues in aquaculture. Appl. Anim. Behav. Sci. 104, 199-235.

Balcombe, J.P., 2006. Laboratory environments and rodents' behavioural needs: a review. Lab. Anim. 40, 217-235.

Balon, E.K., 2004. About the oldest domesticates among fishes. J. Fish Biol. 65, 1-27.

Barcellos, L.J.G., Kreutz, L.C., Quevedo, R.M., da Rosa, J.G.S., Koakoski, G., Centenaro, L., Pottker, E., 2009. Influence of color background and shelter availability on jundia (Rhamdia quelen) stress response. Aquaculture 288, 51-56.

Basquill, S.P., Grant, J.W.A., 1998. An increase in habitat complexity reduces aggression and monopolization of food by zebra fish (Danio rerio). Can. J. Zool. 76, 770-772.

Boissy, A., Arnould, C., Chaillou, E., Desire, L., Duvaux-Ponter, C., Greiveldinger, L., Leterrier, C., Richard, S., Roussel, S., Saint-Dizier, H., Meunier-Salaun, M.C., Valance, D., Veissier, I., 2007. Emotions and cognition: a new approach to animal welfare. Anim. Welf. 16, 37-43.

Broglio, C., Rodriguez, F., Salas, C., 2003. Spatial cognition and its neural basis in teleost fishes. Fish Fish. 4, 247-255.

Broom, D.M., 1991. Animal Welfare: Concepts and Measurement. J. Anim. Sci. 69, 4167-4175.

Broom, D.M., 2007. Cognitive ability and sentience: Which aquatic animals should be protected? Dis. Aqaut. Organ. 75, 99-108.

Brown, C., Laland, K.N., 2003. Social learning in fishes: a review. Fish Fish. 4, 280-288.

Bshary, R., Wickler, W., Fricke, H., 2002. Fish cognition: a primate's eye view. Anim. Cogn. 5, 1-13.

BVET, 2009. Bundesamt für Veterinärwesen. Tierversuchstatistik. http://www.bvet.admin.ch.

Carfagnini, A.G., Rodd, F.H., Jeffers, K.B., Bruce, A.E.E., 2009. The effects of habitat complexity on aggression and fecundity in zebrafish (Danio rerio). Environ. Biol. Fish. 86, 403-409.

Casamitjana, J., 2004. Aquatic Zoos. A Critical Study of UK Public Aquaria in the Year 2004. The Captive Animals' Protection Society.

Chandroo, K.P., Duncan, I.J.H., Moccia, R.D., 2004. Can fish suffer?: perspectives on sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86, 225-250.

Dawkins, M.S., 2003. Behaviour as a tool in the assessment of animal welfare. Zoology 106, 383-387.

Dawkins, M.S., 2006. A user's guide to animal welfare science. Trends Ecol. Evol. 21, 77-82.

Dickens, M., 1955. A Statistical Formula to Quantify the "Spread-of-Participation" in Group Discussion. Speech Monographs 22, 28-30.

Engeszer, R.E., Patterson, L.B., Rao, A.A., Parichy, D.M., 2007. Zebrafish in the Wild: A Review of Natural History and New Notes from the Field. Zebrafish 4, 21-40.

Galhardo, L., Correia, J., Oliveira, R.F., 2008. The effect of substrate availability on behavioural and physiological indicators of welfare in the African cichlid (Oreochromis mossambicus). Anim. Welf. 17, 239-254.

Hamilton, I.M., Dill, L.M., 2002. Monopolization of food by zebrafish (Danio rerio) increases in risky habitats. Can. J. Zool. 80, 2164-2169.

Hughes, B.O., Duncan, I.J.H., 1988. The notion of ethological 'need', models of motivation and animal welfare. Anim. Behav. 36, 1696-1707.

Huntingford, F.A., Adams, C., Braithewaite, V.A., Kadri, S., Pottinger, G., Sandoe, P., Turnbull, J.F., 2006. Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Hutter, S., Penn, D.J., Magee, S., Zala, S.M., 2010. Reproductive behaviour of wild zebrafish (Danio rerio) in large tanks. Behaviour 147, 641-660.

Iwama, G.K., 2007. The welfare of fish. Dis. Aqaut. Organ. 75, 155–158.

Jacobs, J., 1974. Quantitative measurement of food selection. Oecologia 14, 413-417.

Johansen, R., Needham, J.R., Colquhoun, D.J., Poppe, T.T., Smith, A.J., 2006. Guidelines for health and welfare monitoring of fish used in research. Lab. Anim. 40, 323-340.

Kirkden, R.D., Pajor, E.A., 2006. Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. Appl. Anim. Behav. Sci. 100, 29-47.

Kistler, C., Hegglin, D., Würbel, H., König, B., 2010. Structural enrichment and enclosure use in an opportunistic carnivore: the red fox. Anim. Welf. 19, 391-400.

Kortmulder, K., 1981. Etho-Ecology of 17 Barbus Species (Pisces, Cyprinidae). Neth. J. Zool. 32, 144-168.

Lawrence, C., 2007. The husbandry of zebrafish (Danio rerio): A review. Aquaculture 269, 1-20.

Livengood, E.J., Chapman, A., 2007. The Ornamental Fish Trade: An INTRODUCTION with Perspectives for Responsible Aquarium Fish Ownership. Department of Fisheries and Aquatic Sciences, Florida. http://edis.ifas.ufl.edu.

Mason, G., Clubb, R., Latham, N., Vickery, S., 2007. Why and how should we use environmental enrichment to tackle stereotypic behaviour? Appl. Anim. Behav. Sci. 102, 163-188.

Mason, G., Mendl, M., 1993. Why is there no simple way of measuring animal welfare? Anim. Welf. 2, 301-319.

Mason, G.J., 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015-1037.

McClure, M.M., McIntyre, P.B., McCune, A.R., 2006. Notes on the natural diet and habitat of eight

danionin fishes, including the zebrafish Danio rerio. J. Fish Biol. 69, 553-570.

Mendl, M., 2001. Assessing the welfare state. Nature 410, 31-32.

Paull, G.C., Filby, A.L., Giddins, H.G., Coe, T.S., Hamilton, P.B., Tyler, C.R., 2010. Dominance

Hierarchies in Zebrafish (Danio rerio) and Their Relationship with Reproductive Success.

Zebrafish 7, 109-117.

Riehl, R., Baensch, H.A., 1983. Aquarienatlas. Mergus Verlag für Natur- und Tierheimkunde Baensch,

Melle, Germany.

Rose, J.D., 2002. The Neurobehavioral nature of fishes and the question of awareness and pain. Rev.

Fish. Sci. 10, 1-38.

Rotllant, J., Tort, L., Montero, D., Pavlidis, M., Martinez, M., Bonga, S.E.W., Balm, P.H.M., 2003.

Background colour influence on the stress response in cultured red porgy Pagrus pagrus.

Aquaculture 223, 129-139.

Saxby, A., Adams, L., Snellgrove, D., Wilson, R.W., Sloman, K.A., 2010. The effect of group size on

the behaviour and welfare of four fish species commonly kept in home aquaria. Appl. Anim.

Behav. Sci. 125, 195-205.

Shannon, C.E., Weaver, W., 1949. The mathematical theory of communication. University of Illinois

Press, Urbana.

Shyne, A., 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo

mammals. Zoo Biol. 25, 317-337.

Spence, R., Ashton, R., Smith, C., 2007a. Oviposition decisions are mediated by spawning site quality

in wild and domesticated zebrafish, Danio rerio. Behaviour 144, 953-966.

Spence, R., Fatema, M.K., Ellis, S., Ahmed, Z.F., Smith, C., 2007b. Diet, growth and recruitment of

wild zebrafish in Bangladesh. J. Fish Biol. 71, 304-309.

Spence, R., Fatema, M.K., Reichard, M., Huq, K.A., Wahab, M.A., Ahmed, Z.F., Smith, C., 2006. The

distribution and habitat preferences of the zebrafish in Bangladesh. J. Fish Biol. 69, 1435-

1448.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish,

Danio rerio. Biol. Rev. 83, 13-34.

Stolba, A., Woodgush, D.G.M., 1989. The Behavior of Pigs in a Semi-Natural Environment. Animal Production 48, 419-425.

Vascotto, S.G., Beckham, Y., Kelly, G.M., 1997. The zebrafish's swim to fame as an experimental model in biology. Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire 75, 479-485.

Volpato, G.L., 2009. Challenges in Assessing Fish Welfare. Ilar J. 50, 329-337.

Wechsler, B., 2007. Normal behaviour as a basis for animal welfare assessment. Anim. Welf. 16, 107-110.

Williams, T.D., Readman, G.D., Owen, S.F., 2009. Key issues concerning environmental enrichment for laboratory-held fish species. Lab. Anim. 43, 107-120.

Würbel, H., Chapman, R., Rutland, C., 1998. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. Appl. Anim. Behav. Sci. 60, 69-81.

Zar, J.H., 1999. Biostatistical analysis. Prentice Hall, New Jersey, USA.

GENERAL DISCUSSION

General Discussion

Environmental enrichment is a concept that has been widely used in animal husbandry with the aim to improve housing conditions and therefore animal welfare. It is a concept that intends to define cues that enable animals to show their species-specific behaviour, and is often combined with a naturalistic approach that takes into account the animal's natural environment it has evolved in and its natural behavioural repertoire (Shepherdson, 1998). In my thesis, I aimed at testing the concept of environmental enrichment using a naturalistic approach with different taxonomic groups, a mammal species and different ornamental fish species. My studies were based on the two questions "Are animals healthy" and "Do animals have what they want" (Dawkins, 2003). Therefore, to assess the effect of the enrichments, I used behavioural diversity and the absence of abnormal behaviours as proxies for good welfare. Furthermore, using preference tests I tried to evaluate how the study species valued different types of enrichment.

Due to their biology, opportunistic carnivores such as the red fox (*Vulpes vulpes*) provide interesting possibilities to investigate environmental enrichment. Ornamental and laboratory fish are still poorly studied regarding animal welfare, and therefore provide a promising field to apply the concept of environmental enrichment. First, I discuss the importance of a feeding enrichment for opportunistic carnivores that allows animals to exhibit species-specific foraging behaviour, and thus prevent the occurrence of abnormal behaviours. Second, I discuss the importance of structural enrichment to add complexity to an enclosure or an aquarium, and thus providing the animals with an environment resembling their natural habitat. Finally, I provide my conclusion on environmental enrichment and suggest future research questions in connection with preference and motivation testing.

Feeding enrichment

The results of my study on feeding enrichment for an opportunistic carnivore support the evidence that captive animals benefit from species-adequate feeding enrichments. The aim of the feeding enrichment was to simulate the natural situation and therefore to provide food in an unpredictable and time-consuming form. In wild carnivores, the occurrence of their food resources is often unpredictable in time and space, and searching for food accounts for a considerable part of their time budget. In captivity, carnivores are frequently provided with food in an easy accessible form at predetermined schedules, and often only once a day. Thus, foraging is restricted to eating what accounts for the least

part of the temporal and physical effort wild carnivores have to make to get food (Schaller 1978). Such

feeding regimes lack stimuli so that animals cannot exhibit their species-specific behaviour, what may

cause frustration and lead to abnormal behaviours such as stereotypies (Mason, 1993). Opportunistic

carnivores such as red foxes have a wide trophic niche. They browse their home range to find food

and use different foraging strategies to either catch prey or exploit diverse food resources (Lucherini

and Crema, 1994; Contesse et al., 2004). In my study, electronic feeding devices that could be

programmed to provide food at different times and places throughout the day, and independent of

caretakers proved to be useful to simulate the situation in the wild (unpredictability, foraging demand).

This supports the findings in other species where feeders were successful in eliciting appetitive

behaviour (e.g. in wildcats Hartmann-Furter, 2000). Moreover, the different feeding enrichments

offered the possibility to simulate the wide trophic niche of opportunistic carnivores and to provide

different types of food items, such as dead prey or fruit (electronic feeders), but also small food items

(self-service food box, manually scattering food, electronic dispenser) that were time consuming to

find for the foxes. Such combination of unpredictability and time consuming foraging elicited a diverse

foraging behaviour and much exploration and kept the foxes occupied. This suggests a time budget

that is more similar to the situation in the wild (Doncaster and Macdonald, 1997). Opportunistic

carnivores such as foxes are very explorative animals and show a high adaptability to changing

environmental conditions, also reflected by the fact that they increasingly live in urban areas (Gloor et

al., 2001). This may also be a cause for their susceptibility to develop abnormal behaviour such as

stereotypies in captive environments that lack challenging situations and occupational opportunities.

The foxes in my study never developed abnormal behaviours, however, during the conventional

feedings they rested for a high amount of time as a consequence of missing foraging opportunities. In

conclusion, a varied feeding regime that mimics the situation in the wild seems to be most promising

to enrich the feeding situation of captive opportunistic carnivores. Combining different types of

feedings can even enhance the spatial and temporal unpredictability of feeding events, and may also

prevent habituation to the feeding enrichments.

Structural enrichment

The results of the two studies I conducted on structural enrichment support the hypothesis that

enclosure complexity and shelter are important cues in a captive environment. The simulation of

natural habitats to provide animals with biologically relevant information is increasingly implemented in

zoos or wildlife parks (Robinson, 1998), whereas in laboratory or pet animal housing such as in aquaria more practical or aesthetical values predominate. Red foxes and small cyprinids have evolved in cover rich habitats, and they furthermore suffer a high predation risk in their natural environment (Kortmulder, 1981; Lucherini et al., 1995; Spence et al., 2008). In captivity, animals usually do not suffer from predation, however disturbances by human activity may influence the animals' behaviour. Caretakers or the public in zoos and public places, and the owners of pet animals in private homes are potential sources of irritation. Therefore, structural enrichment can provide shelter from outside disturbances (Carlstead, 1991; Frézard and Le Pape, 2003). I found in foxes that they preferred areas with structures that mimicked natural features such as hedges or thickets when they were browsing the enclosure (chapter 2). Also in fish, I found a preference for the structured compartment containing vegetation and cover (chapter 3 and 4). Whereas in foxes the netlike arrangement of structures seemed to be crucial to make space accessible, in fish the distribution of structures in the water column proved to be if high importance.

Aggression and competition between conspecifics are part of the natural behavioural repertoire in group-living species. However, restricted conditions in captivity may cause increased aggressive interactions. Both, red foxes and the different cyprinid species live in groups and form dominance hierarchies. Whereas the red foxes form family groups with one male and one female being the dominant individuals (Cavallini, 1996), the different barbs species and zebrafish rather form anonymous groups that aggregate to forage and for reproduction (Kortmulder, 1981; Spence et al., 2008). The foxes showed some socio-negative behaviour, however, I never observed extended aggressive behaviour between individuals. Most of the fish exhibited socio-negative behaviour in the structured environment, which probably was due to territorial behaviour and different mating tactics. Nevertheless, structural enrichment can enable subordinate individuals to escape aggression from conspecifics and therefore fulfils an important function as visual barrier. In conclusion, structural enrichment that mimics the natural environment, i.e. the nature and the arrangement of the structures, seems to be most useful for a species-specific furnishing of enclosures and aquaria.

Environmental enrichment has rarely been tested in laboratory fish. I found in my study that zebrafish generally showed a preference for a complex environment. Zebrafish are increasingly used as animal models in research (Vascotto et al., 1997). In laboratories, they are usually held in mixed sex groups

in small barren tanks (pers. observation). Research in other species revealed that laboratory animals suffer from inadequate housing (Würbel et al., 1998), and that brain development is influenced by environmental conditions (Lewis, 2006). As fish are animals with a sophisticated behaviour, and studies on fish brains show that cognitive abilities, e.g. spatial cognition, are based on neural mechanisms homologous to those of mammals and birds (Broglio et al., 2003), fish probably also suffer from restricted housing conditions in laboratories. Moreover, as in other laboratory animals (Sherwin, 2004) the question arises also in fish if current standard housing conditions impair the validity of scientific results.

Conclusions

The results of my study on feeding and structural enrichments support the hypothesis that the concept of environmental enrichment can help to establish a species-adequate housing with a stimulating environment and prevent behavioural disturbances, and thus enhance animal welfare. The concept of environmental enrichment can be applied on a wide range of species. Using a naturalistic approach allows to identify the species-specific behavioural and ecological needs of captive animals. The combination of feeding and structural enrichment can add environmental complexity and relevant biological information to a captive environment. Whereas a varied feeding enrichment can influence the time budget and behavioural diversity, structural enrichment can influence space use and fulfils various different functions such as cover and shelter, visual barriers, landmarks, partitions, food or resources for reproduction. It has been proven that the environment influences brain development (Würbel, 2001). Therefore, it is essential that animals be raised in complex and stimulating environments in order to prevent behavioural disturbances, not least animals that are used in research. The natural and simple environmental enrichments I used for the fish worked well. However, for laboratory use they would be difficult to implement as in lab conditions environmental enrichment has primarily to be easy to handle and hygienic. Nevertheless, artificial structures providing natural stimuli might fulfil the purpose of enrichment, comparable to the enrichment of lab mouse housing (Olsson and Dahlborn, 2002).

Future research

Behavioural measures can yield valid information about preferences of animals. However, physiological measures in addition to behavioural measures would be needed to confirm enhanced

welfare in presence of environmental enrichment (Mason and Mendl, 1993). Non-invasive methods to measure stress hormones in faeces of terrestrial animals and in water of aquatic animals, respectively, provide interesting possibilities to investigate the influence of environmental enrichment on welfare. Furthermore, more sophisticated preference tests using measures of strength of preference or changing preference in the presence of further resources would allow more detailed information about which resources are really valued by animals (Kirkden and Pajor, 2006). Combining welfare (behavioural and physiological measures) and motivational measures, i.e. how much do animals work for access to a resource, would provide evidence which enrichments specifically enhance welfare (Mendl, 2001). In my study, the red foxes but also the cyprinid species showed considerable learning skills, and would therefore be interesting study species for motivation testing.

REFERENCES

Broglio, C., Rodriguez, F., Salas, C., 2003. Spatial cognition and its neural basis in teleost fishes. Fish Fish. 4, 247-255.

Carlstead, K., 1991. Husbandry of the Fennec fox *Fennecus zerda*: Environmental conditions influencing stereotypic behaviour. Int. Zoo. Yearb. 30, 202-207.

Cavallini, P., 1996. Variation in the social system of the red fox. Ethology Ecology & Evolution 8, 323-342.

Contesse, P., Hegglin, D., Gloor, S., Bontadina, F., Deplazes, P., 2004. The diet of urban foxes (*Vulpes vulpes*) and the availabilty of anthropogenic food in the city of Zurich, Switzerland. Mamm. Biol. 69, 81-95.

Dawkins, M.S., 2003. Behaviour as a tool in the assessment of animal welfare. Zoology 106, 383-387.

Doncaster, C.P., Macdonald, D.W., 1997. Activity patterns and interactions of red foxes (*Vulpes vulpes*) in Oxford city. Journal of Zoology 241, 73-87.

Frézard, A., Le Pape, G., 2003. Contribution to the Welfare of Captive Wolves (Canis lupus lupus): A Behavioral Comparison of Six Wolf Packs. Zoo Biol. 22, 33–44.

Gloor, S., Bontadina, F., Hegglin, D., Deplazes, P., Breitenmoser, U., 2001. The rise of urban fox populations in Switzerland. Mamm. Biol. 66, 155-164.

Hartmann-Furter, M., 2000. A species-specific feeding technique for European wildcats (*Felis s. sylvestris*) in captivity. Säugetierkd. Inf. 4, 567-575.

Kirkden, R.D., Pajor, E.A., 2006. Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. Appl. Anim. Behav. Sci. 100, 29-47.

Kortmulder, K., 1981. Etho-Ecology of 17 Barbus Species (Pisces, Cyprinidae). Neth. J. Zool. 32, 144-168.

Lewis, M.H., Presti, M.F., Lewis, J.B., Turner, C.A. , 2006. The Neurobiology of Stereotypy I: Environmental Complexity, In: Mason, G., Rushen, J. (Ed.), Stereotypic Behaviour in Captive Animals: Fundamentals and Applications for Welfare, 2nd ed.

Lucherini, M., Crema, G., 1994. Seasonal variation in diet and trophic niche of the red fox in an alpine habitat. Zeitschrift für Säugetierkunde 59, 1-8.

Lucherini, M., Lovari, S., Crema, G., 1995. Habitat use and ranging behaviour of the red fox (*Vulpes vulpes*) in a Mediterranean rural area: Is shelter availability a key factor? Journal of Zoology 237, 577-591.

Mason, G., 1993. Forms of stereotypic behaviour, In: Lawrence, A.B., Rushen, J. (Eds.), Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare, CAB International, Wallingford, pp. 7-40.

Mason, G., Mendl, M., 1993. Why is there no simple way of measuring animal welfare? Anim. Welfare 2, 301-319.

Mendl, M., 2001. Assessing the welfare state. Nature 410, 31-32.

Olsson, I.A.S., Dahlborn, K., 2002. Improving housing conditions for laboratory mice: a review of 'environmental enrichment. Lab. Anim. 36, 243-270.

Robinson, M.H., 1998. Enriching the lives of zoo animals, and their welfare: where research can be fundamental. Anim. Welfare 7, 151-175.

Shepherdson, D., 1998. Tracing the Path of Environmental Enrichment in Zoos, In: Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), Second nature: Environmental Enrichment for Captive Animals, Smithsonian Institution Press, Washington, pp. 1-14.

Sherwin, C.M., 2004. The influences of standard laboratory cages on rodents and the validity of research data. Anim. Welfare 13, S9-S15.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, Danio rerio. Biol. Rev. 83, 13-34.

Vascotto, S.G., Beckham, Y., Kelly, G.M., 1997. The zebrafish's swim to fame as an experimental model in biology. Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire 75, 479-485.

Würbel, H., 2001. Ideal homes? Housing effects on rodent brain and behaviour. Trends Neurosci. 24, 207-211.

Würbel, H., Chapman, R., Rutland, C., 1998. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. Appl. Anim. Behav. Sci. 60, 69-81.

ACKNOWLEDGEMENTS

problems! My thanks go also to Fabio who was always ready to help me to find solutions to any problem with his extraordinary analytic mind and scientific experience. Thank you, Fabio, for being such a great motivator. Moreover, Dani and Fabio, our experts in aquaristics, encouraged me to enter a new dimension and to work with fish; and after being a bit sceptical at first, I now fully agree with them that fish are as fascinating animals as mammals. However, Dani, in one point you are still wrong, and neither the numerous discussions during our legendary coffee breaks, nor all the psychological influencing and votes will change this fact: Zebrafish are BLUE-striped! (I am sorry, Dani, but cheers for the beer…!). Thanks to Annie, Adrian and Thomas for many laughs, jokes and rich discussions about animal housing and animal welfare. I also thank Kuno for helping me installing all the aquaria, and the trainee Stephie helping me to empty and clean all the aquaria in the end, and Marco who took some nice photographs of the fish, which is not an easy task.

I would like to express my gratitude to Christian Stauffer, head of the Wildnispark Zürich, for giving me the possibility to study foxes at Langenberg Wildlife Park and to use all the facilities there. He offered me the unique opportunity to contribute to the project of building a new, species-adequate enclosure for foxes. The approach of first doing experiments in a test enclosure and then implement the results in a newly built enclosure, is rather unusual in applied zoo biology studies. All the more, I consider myself very lucky to have been able to conduct this project, and it was a rich experience to work at Langenberg. I would also like to thank Res Reifler and Mirjam Würsch for their friendly support, Urs von Riedmatten, Andi Peemöller, and Albert Winzeler for their great help in establishing the test enclosure according to my ideas and needs, not forgetting the amazing hide they built especially for the purpose of observing the foxes. I owe special thanks to Andi who built the simple but genial self-service food box, and to Iann Romer who rebuilt the electronic dispenser with a lot of technical imagination and skills.

I am grateful to Marianne Hartmann who encouraged me to do this PhD project and for the discussion about the experimental procedure in testing species-adequate housing.

I am also indebted to Josh van Buskirk and Professor Uli Reyer. Despite the desperate space situation at the university they offered me a part in one of the animal houses where I could install all my aquaria and conduct my experiments. Thanks also to Professor Stephan Neuhauss and Kara Dannenhauer

who kindly supplied me with the various strains of zebrafish and information about laboratory housing conditions.

I thank the group of "Verhaltensbiologie" for any assistance and, although I turned up rather seldom, the nice chats. Many thanks go to Regula Scherrer and her successor Marianne Köpfler for completing all the administrative work in such a straightforward manner. Very special thanks to Monika Schiess for her encouragements and the unfortunately rare, but nevertheless intensive discussions about animal housing and many more things...

My deepest thank go to all my friends who always listened with great interest and humour to this crazy biologist and her stories, and for all the beautiful moments we experienced together that make things easier in life!
And last but not least, a big hug to you, Amedeo, for all your support all these years, for your humour, for all the funny cartoons to cheer me up, for your ingenuity, and for all the time we spent together.

CURRICULUM VITAE

PERSONALIEN

Name	KISTLER
Vorname	Claudia
Geburtsdatum	18.9.1967
Bürgerort	Zürich / Reichenbach SZ

AUSBILDUNG

1990 -1993   **AKAD, Eidgenössische Matura Typus D, Abteilung für Berufstätige**

1993 - 2000   **Studium am Zoologischen Institut der Universität Zürich**

1998 - 2000   **Diplomstudium am Zoologischen Institut der Universität Zürich**
Titel der Diplomarbeit: „ Der Einfluss der Vertrautheit zwischen Muttersauen (*Sus scrofa domesticus*) auf das Fremdsaugen"
Leitung: Frau Prof. Dr. Barbara König

2004 - 2011   **Promotionsstudium am Institut für Evolutionsbiologie und Umweltwissenschaften der Universität Zürich**
Titel der Dissertation „ The Importance of of Environmental Enrichment for Explorative Animals - Red Foxes (*Vulpes vulpes*) and Cyprinid Fish as Case Studies "
Leitung: Frau Prof. Dr. Barbara König

PUBLIKATIONEN

2011   Kistler, C., Hegglin, D., Würbel, H., König, B. Preference for
                              structured environment in zebrafish (*Danio rerio*) and checker barbs
                              (*Barbus oligolepis*). In press.

2010   Kistler, C., Hegglin, D., Würbel, H., König, B. Structural enrichment
                              and enclosure use in an opportunistic carnivore: the red fox. Animal
                              Welfare 19, 391-400.

2009   Kistler, C., Hegglin, D., Würbel, H., König, B. Feeding enrichment in
                              an opportunistic carnivore: The red fox. Appl. Anim. Behav. Sci. 116,
                              260–265.

2002   Speiser B., Kistler C. Field tests with a molluscicide containing iron
                              phosphate. Crop Protection, 21: 389-394.

1998   Ward, P.I., Mosberger, N., Kistler, C., Fischer, O. The relationship
                              between popularity and body size in zoo animals. Conservation
                              Biology 12, 1408-1411.