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Consistency of individual differences in behaviour of the lion-headed cichlid, *Steatocranus casuarius*

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Abstract

The development of individual differences in behaviour in a novel environment, in the presence of a strange fish and during aggressive interactions with a mirror-image was studied in the lion-headed cichlid (*Steatocranus casuarius*, Teleostei, Cichlidae). No consistency in behaviour was found at 4–5.5 months of age. However, behaviours scored in situations involving a discrete source of stress (a strange fish or conspecific) become significantly consistent at the age of 12 months. At 4–5.5 but not 12 months of age, larger individuals approached and attacked the strange fish significantly more than smaller ones. These patterns may be associated with development and integration of motivational systems and alternative coping strategies.

Keywords: Aggression; Consistency; Individual differences; Exploratory behaviour; Temperament

1. Introduction

Individual differences and alternative strategies have been documented in animals of many species. It is known that they may be adaptive and arise within the population for a variety of reasons (Slater, 1981; Clark and Ehlinger, 1987; Magurran, 1993; Wilson et al., 1994). Although much research has been devoted to the study of alternative strategies and their adaptive function (see Dunbar, 1982; Clark and Ehlinger, 1987; Benus et al., 1991; Magurran, 1993 for reviews), relatively less effort has been directed to the analysis of the development of individuality. Moreover, while various aspects of its ontogeny have been studied in mammals (e.g. Fox, 1972; MacDonald, 1983; Stevenson-Hinde, 1983; Lyons et al., 1988; Loughry and Lazari, 1994), the development of individual differences in behaviour, especially in consistent behavioural traits, is almost an open question in other species, such as fishes. Even though a recent longitudinal study (see Francis, 1990) revealed significant consistency and continuity of aggressiveness in the Midas cichlid (*Cichlasoma citrinellum*), no other behaviours except aggression were examined.

In this investigation we analysed stability of individual differences in behaviour of the lion-headed cichlid, *Steatocranus casuarius*, a common

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inhabitant of rocky habitats in rivers of Western Africa: lower Congo River and its tributaries (Linke and Staeck, 1994). We attempted to determine to what extent individual behavioural differences are consistent in situations involving novelty and aggression. These behavioural domains are stressful, may implicate general alternative coping strategies (strategies to cope with a wide range of environmental demands and stressors, see Benus et al., 1991; Verbeek et al., 1994), and it is known that behavioural consistency increases in stressful situations (e.g. Suomi, 1983; Gerlai and Csányi, 1990; Alados et al., 1996; Budaev, 1997; Budaev et al., 1999).

2. Materials and methods

2.1. Subjects and maintenance conditions

The subjects were aquarium-bred individuals of the lion-headed cichlid, which were obtained from a private breeder. Newly emerged fry were raised in a group of approximately 60 individuals in a 0.6 m^3 tank. At the age of 3.5 months, 12 individuals (standard length 28–36 mm) were randomly selected for the first experimental group. They were transferred to three identical housing aquariums (60 × 30 × 20 cm), four subjects in each. The fish were allowed 2 weeks for adaptation to the maintenance conditions, after which the experiments were started. The fish were kept in these groups for all duration of the study. Thus, they received normal social experience, hardly possible if they were housed in isolation. Possible maintenance in individual chambers separated by transparent walls, allowing only a visual contact, was also undesirable because it could alter aggressive behaviour of cichlid fish (see Fernö, 1986).

All housing aquariums contained removable plastic constructions (several grey plastic plates attached to each other) allowing the fish to hide within. During the study, individuals were fed daily with live and frozen bloodworms (*Chironomus* sp. larvae) and commercial flake food. The water temperature in both the housing aquariums and experimental apparatuses was maintained at 24–26°C and the dark–light period was 10:14 h. Sex could not be reliably determined in the non-reproductive fish used in the study (see Linke and Staeck, 1994). We used hot branding of the caudal fin membrane (cauterising small holes in it) to mark the experimental subjects. This method of marking does not cause much trauma to small fish of comparable size (see McNicol and Noakes, 1979).

2.2. Tests and procedures

The fish were tested in three tests, as described below. In each test subjects were selected in a randomised order. All observations were conducted through a small window made in a screen separating the experimental apparatus from the observer to prevent disturbances of the fish. Previous studies indicated that individual differences in the behaviour of adult guppies (Budaev, 1997) and convict cichlids (Budaev et al., 1999) in similar tests are consistent across situations and reflect underlying fearfulness and coping strategies.

2.2.1. Open field tests

Open field tests were conducted in an empty hexagonal tank, 0.9 m in diameter, with the water level at 8 cm. Initially an individual was gently released into a white bottomless opaque plastic cylinder (the start-box) for 5 min to ensure that it acclimated after the handling. The start-box was necessary because the fish exhibited jerking immediately after release. The start-box was then lifted and the percentage of time which the fish moved in any direction (locomotion score) was recorded.

2.2.2. Strange fish tests

Strange fish tests were performed in an aquarium (60 × 30 × 20 cm) with three consecutive compartments. The ‘home’ compartment was separated from the ‘inspection’ compartment by an opaque partition with a sliding door (6 × 6 cm; at 1 cm above the floor), whereas the third compartment, containing a strange fish (convict cichlid, *Archocentrus* (*Cichlasoma*) *nigrofasciatum*, standard length 64–65 mm), was located behind a transparent glass wall. It also contained a grey plastic fold, preventing the convict cichlid from hiding in a corner. Two small stones creating a sheltering place, were placed in the inspection compartment near the opaque partition with the door, to attract the experimental subject there. Preliminary observations indicated that, in absence of the stimulus fish, individuals preferred to spend the most time in proximity of this shelter. The convict cichlid was always larger than the tested lion-headed cichlid but too small to be a predator, thus providing relatively unthreatening stimuli.

During testing, an individual lion-headed cichlid was gently transferred to the start-box placed into the home compartment with the door closed. The fish was given 6 min to recover from capture and transfer, the start-box was elevated and the behaviour of the fish was observed for 6 min (first recording session). The door was then opened and, as soon as the individual entered the inspection compartment, the second recording session begun, which also continued for 6 min. In both the first and the second recording sessions of this test we recorded locomotion (the percentage of time which the tested fish moved in any direction). Also, the percentage of time which the experimental subject spent inspecting the strange fish and the percentage of time it spent in proximity of the strange fish (<13 cm from the glass partition) were recorded in the second session. Inspections were defined as apparent approaches to the convict cichlid followed by more or less prolonged visual fixations on it.

2.2.3. Mirror tests

Mirror tests were administered in an aquarium measuring $30 \times 30 \times 20$ cm with a mirror (30×20 cm) attached to it outside of the wall. A sheet of semi-opaque plastic film could be placed in front of the mirror. It was sliding and could be gently pulled away using an attached cord. Two stones were placed in the aquarium at its side opposite to the mirror to attract the experimental subject there. Preliminary observations indicated that the fish did not perform any aggressive behaviours and preferred to be in proximity of the stones when the mirror was closed by the film.

The test was administered as follows. First, an individual was gently released into the test apparatus when the mirror was closed with the semi-opaque film. After 10 min was allowed for exploration and adaptation, the film was removed. As soon as the fish approached the mirror to a distance of, approximately, one body size, the recording session was started, which continued for 6 min. We recorded locomotion, as well as the percentage of time devoted to aggressive displays and bites directed to the mirror image (total aggression score). We also recorded the percentage of time which the tested fish spent in proximity of the mirror (<13 cm).

2.3. Experimental design

The experimental design used in this study is schematised in Fig. 1. All three tests described above were administered to the first group of fish ($N=12$) at the age of 4 months, with 1 week between test intervals. Following the open field test, the fish were marked for individual recognition and their standard length was measured. They were re-tested in the same three tests 1 month after the last test of the previous series using identical procedures. Because the tags became poorly distinguishable at this time (as we tried to minimise damage to the caudal fins), we repeated the marking procedure.

The experimental subjects were maintained in the housing aquariums for 6 months. During this period, intense growth forced us to reduce the group size to nine individuals (one individual was randomly removed from each housing aquarium). In addition, a disease outbreak caused a further loss of two individuals, resulting in a sample of seven fish. To accommodate for the subjects loss, a second group of seven naive individuals selected from the same population was formed. It also served as a control for possible effect of repeated testing and prior housing conditions (before entering into experiments, the second group was still maintained in a large conspecific group in a 1.8 m^3 tank). During the following period, the second group was housed in the same conditions as the first group.

These 14 individuals were tested when their age was 12 months and re-tested at 13.5 months of age using procedures identical to those applied earlier. Thus, at the end of the experiment there were two groups of test–retest pairs separated by a 6 month period (Fig. 1). However, it was impossible to trace individual identity of the fish over the 6 month period because permanent tags would cause a significant injury to small individuals and the previously applied caudal hot-branding marks completely disappeared, about 2 months after tagging.

2.4. Statistical analysis

We used nonparametric statistical methods for the data analysis: Spearman rank correlation coefficient with exact P values based on Monte Carlo estimation and randomisation tests (Manly, 1991). All significance levels reported are two-tailed.

3. Results and discussion

Preliminary analysis indicated that there were no significant differences between the two groups of lion-headed cichlids in most behavioural units. However, the second group of fish tended to be less aggressive ($P = 0.045$, randomisation test) and more active ($P = 0.015$, randomisation test) than the first group in the mirror test 3 (which was first for this group). Also, between-group differences in the percentage of time spent inspecting the strange fish in the strange fish test 3 and in the time spent near the mirror in the mirror test 3 approached significance (respectively, $P = 0.097$ and $P = 0.086$, randomisation test). There were no differences in length between the two groups of fish, neither at 12 nor 13.5 months of age (respec-

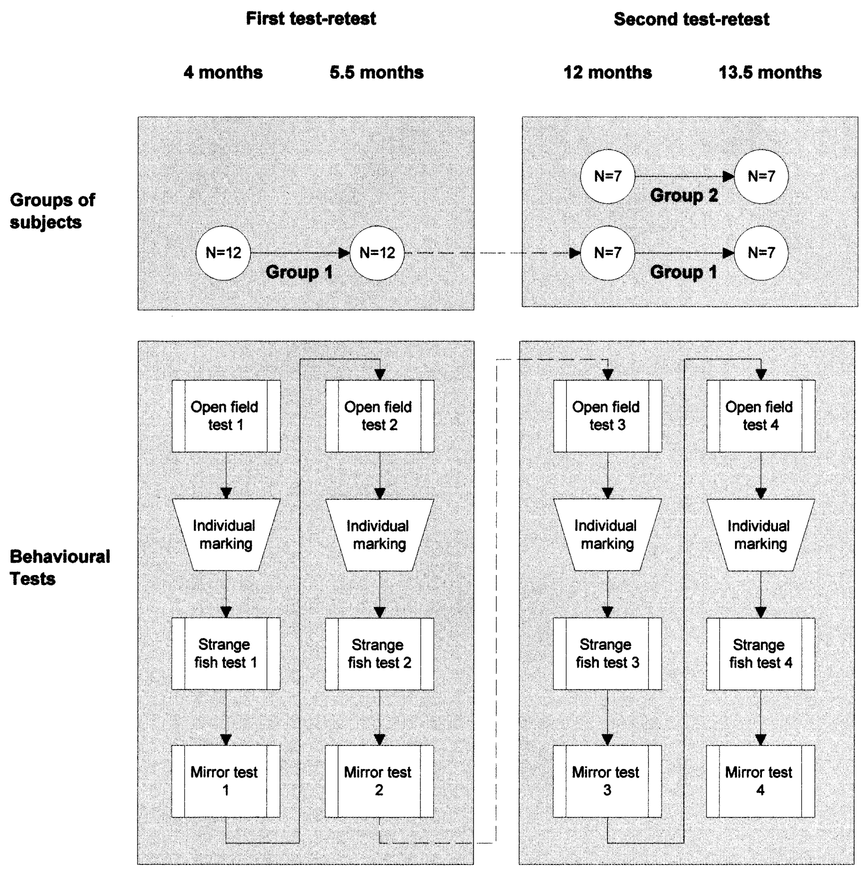

Fig. 1. Scheme of the sequence of tests (1 week time interval between consecutive tests on the vertical). The upper panel depicts groups of subjects, the lower panel shows the sequence of tests.

Table 1
Spearman correlation coefficients between behavioural measures at adjacent test–retest points

Behaviour unit	First test–retest, 4–5.5 m ($N=12$)		Second test–retest, 12–13.5 m ($N=14$)	
	R_s	p	R_s	p
Open field test				
Locomotion	−0.07	0.837	0.13	0.645
Strange fish test				
Locomotion[a]	0.19	0.562	−0.07	0.810
Locomotion[b]	−0.33	0.293	0.78	0.001**
Inspection[b]	0.01	0.974	0.54	0.049*
Time near strange fish[b]	0.17	0.590	0.56	0.040*
Mirror test				
Locomotion	0.42	0.174	0.27	0.365
Total aggression	0.13	0.670	0.64	0.015*
Time near mirror	0.31	0.325	0.66	0.013*

[a] First recording session.
[b] Second recording session (with convict cichlid). *$P<0.05$; **$P<0.01$.

tively, $P=0.568$, $P=0.549$, randomisation test). Furthermore, identical patterns of correlations were found in both groups. Thus, it was justified to pool the data of the two groups.

3.1. Behavioural consistency

Table 1 presents Spearman correlation coefficients between behavioural measures separated by 1.5 months time intervals. It is seen that individual differences in behaviour were not consistent at 4–4.5 months of age, but behavioural stability was present when the lion-headed cichlids reached 12–13.5 month of age.

These results agree with the data obtained in other species. For example, Francis (1990) observed that individual differences in mirror test aggression scores in the Midas cichlid become significantly more consistent with age. Also, in several mammalian species it was shown that individual differences in behaviour are not consistent early in ontogeny, but increase in adults (e.g. MacDonald, 1983; Loughry and Lazari, 1994; see also Hahn et al., 1990), and the same trend was observed in humans (Plomin, 1986). We speculate that this general pattern may be associated with development and integration of motivational systems (Hogan, 1988) and coping strategies (Benus et al., 1991). Yet, certain studies (e.g. Verbeek et al., 1994 on great tits) indicated that individual differences are not necessarily unstable early in ontogeny.

Several investigations showed that consistent individual differences become pronounced in stressful situations (e.g. Suomi, 1983; Gerlai and Csányi, 1990; Alados et al., 1996; Budaev, 1997; Budaev et al., 1999). For example, behavioural complexity or randomness tend to be particularly pronounced in non-threatening situations and could mask possibly consistent individual differences (see Alados et al., 1996; Budaev, 1997). In our study, consistent become only behaviours scored in situations involving a discrete source of stress, such as the presence of an unfamiliar fish and a mirror image 'conspecific'. Also, because there were no significant correlations between aggression and strange fish inspection (all P values >0.1), it is unlikely that approach to and inspection of the strange fish primarily reflected aggressive motivation.

3.2. Body size and behavioural variables

The fish body length proved to be highly consistent over time: the first test–retest correlation was 0.85 ($N=12$, $P<0.0001$) and the second test–retest correlation was 0.96 ($N = 14$, $P < 0.0001$). There was an interesting age-related pattern of relationships between body size and behaviour variables. During the first test period, the time spent near the strange fish and near the mirror correlated with the body size ($R_s = 0.60$, $P = 0.035$, $R_s = 0.67$, $P = 0.022$ respectively). One month later, when the tests were repeated, the total aggression score, the time spent near the mirror and the time spent near the strange fish also significantly correlated with the body length ($R_s = 0.86$, $P < 0.001$, $R_s = 0.63$, $P = 0.028$, $R_s = 0.62$, $P = 0.035$, respectively). However, at the age of 12 and 13.5 months, the correlations between behavioural variables and body size disappeared (all P values > 0.2).

Thus, large body size could induce higher boldness and aggressiveness at 4–5 months of age, presumably through its mediating effects on early social status. Smaller individuals may be relatively more stressed and therefore had shyer and less aggressive 'moods'. But consistent behavioural traits would become more important later in ontogeny, reducing the correlation. An alternative hypothesis, that bolder or more aggressive fish might have higher competitive abilities, which could subsequently bring about their faster growth (see Huntingford et al., 1990), is less likely because individual differences in behaviour were initially inconsistent. Further studies are required to distinguish between these hypotheses.

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