Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*

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Individual common carp *Cyprinus carpio* were screened repeatedly for risk taking (rate of exploration of a novel, potentially dangerous environment) and for competitive ability (success in gaining access to a spatially restricted food source). Marked differences in behaviour were evident, and significant consistency in individual responses across trials was found for both risk taking and competitive ability. In addition, there was a significant positive relationship between individual performance in these two contexts, with fish that explored more quickly in the novel environment tending to be among the first to gain access to restricted food. In two follow-up studies, resting metabolic rate, blood lactate and glucose and the expression of the cortisol receptor gene in the head kidney and brain were compared in fish from the two extremes of the risk-taking spectrum. Mass-specific metabolic rate was significantly higher in risk-taking than in risk-avoiding fish, while plasma lactate and glucose concentrations and expression of the cortisol receptor gene were lower. It was concluded that a behavioural syndrome based on boldness and aggression exists in *C. carpio*, as it does in many other animals, and that this is associated with differences in metabolic and stress physiology (down to the genomic level) similar to those described in animals with different coping strategies.

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Key words: competitive ability; cortisol receptor; metabolic rate; risk taking.

INTRODUCTION

In a range of vertebrate species, suites of behavioural traits have been identified that co-vary at the individual level; these are referred to as behavioural syndromes. For example, a common finding is that risk taking in response to potential danger is correlated with aggressiveness when competing for resources (Sih *et al.*, 2004). In addition, individual animals often differ strikingly in physiological responses to challenge or show different coping strategies (Korte *et al.*, 2005; Koolhaas *et al.*, 2007). So-called proactive individuals typically show an active (fright and flight),
adrenaline-based response to various challenges, are aggressive towards competitors, take risks in the face of potential danger and are relatively inflexible, tending to form behavioural routines. In contrast, so-called reactive individuals show a passive (freeze and hide), cortisol-based response to challenge, avoid risk (including fights), but are responsive to and flexible about environmental change.

Consistent individual variability in risk taking linked in a behavioural syndrome with aggressiveness has been documented for a number of species of fishes, including three-spined sticklebacks *Gasterosteus aculeatus* L. (Huntingford, 1976), although this association can depend on the rearing environment (Bell & Sih, 2007), brown trout *Salmo trutta* L. (Sundstrom et al., 2004), rainbow trout *Oncorhynchus mykiss* (Walbaum) (Schjolden et al., 2005a, b) and grayling *Thymallus thymallus* (L.) (Salonen & Peuhkuri, 2006). As far as physiological variability is concerned, *O. mykiss* provides the best-documented example of coping strategies in fishes, although Bell et al. (2007) report correlations between individual risk-taking behaviour and brain biochemistry in *G. aculeatus*. In *O. mykiss*, strains selected for high and low cortisol responsiveness to a standardized stressor also differ in their behaviour (Øverli et al., 2005; Schjolden & Winberg, 2007). Fishes from the low responsive strain adapt more quickly to a novel environment than do those from the high responsive strain and also tend to become socially dominant in pair-wise tests (Øverli et al., 2005), although again this association can depend on recent environmental conditions (Ruiz et al., 2008).

The general literature on coping strategies includes discussion of differences in energy metabolism, with proactive animals often adopting an energetically expensive strategy and reactive animals being energetically conservative (Korte et al., 2005). Stamps (2007) and Biro & Stamps (2008) argue that consistent individual differences in boldness and aggression as well as correlations between these traits may arise through a growth–mortality trade-off. According to this view, fast-growing individuals show both physiological and behavioural adaptations for efficient growth, including high metabolic rate and, in terms of behaviour, a tendency to take risks while foraging and to fight over food; those adopting a slow-growing trajectory will show the opposite traits, hence the correlation between boldness and aggression. Careau et al. (2008) also argue that metabolic rate may be linked to an individual behavioural profile. According to one model (the performance model), a positive relationship is predicted between resting metabolic rate and activity or aggressiveness, an active life style requiring well-developed machinery for acquiring and processing food, which will have higher than average maintenance costs (Daan et al., 1990). There have been no direct comparisons of metabolic rate in risk-taking and risk-avoiding fishes, although differences in metabolic rate have been suggested as the reason for the observed association between risk taking and body size described in poeciliids (Brown & Braithwaite, 2004; Brown et al., 2007). In addition, in several fish species, individuals with the higher resting metabolic rate are more likely to win pair-wise fights than are opponents with lower metabolic rates (Metcalfe et al., 1995; Yamamoto et al., 1998; McCarthy, 2001). Overall, however, there is relatively little information for fishes about the relationship between metabolic rate and risk taking or about physiological correlates of individual variability in risk taking. In the present study, a series of studies on common carp *Cyprinus carpio* L. were undertaken to fill this gap.
Individual variation in risk taking in a potentially dangerous environment has been documented for crucian carp *Carassius auratus langsdorffii* Temminck & Schlegel and goldfish *Carassius auratus* (L.) (Yoshida *et al*., 2005), and it is of interest to establish whether the same is the case for *C. carpio*. Within schools of *C. carpio*, there is competition for food that mostly takes the form of scramble competition, though aggressive interactions have been observed in small groups of *C. auratus* foraging on clumped food (Stenberg & Persson, 2005). It is also of interest to determine whether individual *C. carpio* vary in their effectiveness in scramble competition and, if so, whether differences in risk taking and competitive ability are organized into a behaviour syndrome. Metabolic rate in *C. carpio* changes in response to environmental conditions, including salinity (De Boeck *et al*., 2000) and diet (Siddhuraju & Becker, 2002). Even for fish of a similar size in identical conditions, however, there is variability in metabolic rate and it is of interest to see whether some of this can be explained by risk-taking phenotype. The physiological stress response is well characterized in *C. carpio* and shows variability at the individual level that is at least partially inherited (Tanck *et al*., 2001, 2002). It is therefore also of interest to establish whether such physiological variation is related to any observed differences in risk taking at a behavioural level. During a study of differential gene expression, plasma lactate and glucose levels as well as cortisol receptor (CR) expression (known to be related to plasma cortisol levels in this species, Weyts *et al*., 1998) were measured in *C. carpio* with different risk-taking phenotypes. These data are included here.

With this background, the specific aims of the three experiments reported here were: (1) to quantify individual variability in risk taking in *C. carpio* exposed to a novel, potentially dangerous environment (experiment 1); (2) to quantify the performance of individual *C. carpio* in competition for a spatially restricted food source, to examine repeatability of these two traits (experiment 1); (3) to investigate the relationship between risk taking and competitive performance (experiment 1); and (4) to compare resting metabolic rate (experiment 2) and indicators of stress responsiveness (cortisol receptor expression in the head kidney, experiment 3) in *C. carpio* from the extremes of the risk-taking continuum.

**MATERIALS AND METHODS**

**EXPERIMENT 1: A BEHAVIOURAL SYNDROME IN CYPRINUS CARPIO?**

**Fish and general husbandry**

*Cyprinus carpio* [mean ± S.E. total length (**LT**) = 64.7 ± 1.22 mm] were obtained from Murray Aquatics, Glasgow (www.murrayaquatics.co.uk) and were transported to Glasgow University’s Scottish Centre for Ecology & the Natural Environment (SCENE). They were held in groups in a re-circulating system at an average temperature of 18°C and fed daily to satiation on chironomid larvae (bloodworm *Chironomus* sp.) from Gamma Frozen Foods, Tropical Marine Centre (www.tmc-ltd.co.uk).

**Screening for risk taking**

Fish were screened for risk taking using a variation of the well-established novel-environment test, which has been extensively used on a range of species of fishes (Huntingford &
Isolated *C. carpio* showed behavioural signs of extreme stress, which was deemed likely to obscure any underlying differences in response to risk. Fish were therefore tested in small groups, having been individually marked using alcian blue dye (U.K. Home Office licence number 60/2930). The screening tank was $100 \times 40 \times 40$ cm, with a water depth of 15 cm. Temperature was matched to that of the holding tanks ($c. 18^\circ$ C). At one end of the screening tank, there was an enclosed, darkened settling chamber (25 cm in length), from which a plastic tunnel (11.5 cm in length and 7 cm in diameter, with its base 3 cm from the bottom of the tank) formed an exit into the main section of the tank. The diameter of the exit tube was such that only one fish could emerge at a time. The opening to the tunnel was fitted with a removable, opaque plastic cover. The main section of the tank had a sandy substratum, was illuminated from above and represented a novel, potentially dangerous environment for exploration. A glass beaker (14 cm in diameter and 17 cm in height, containing $c. 2.1$ l of water) was placed in the centre of the main section, 32 cm from the tunnel and visible through the tunnel once the cover had been removed. Pilot studies showed that most fish would emerge within the allocated period with a good spread of emergence times if exposed to the sight and smell of food. Therefore, before each test, defrosted bloodworms were placed in the beaker, with an air stone to create turbulence within the beaker and movement in the bloodworms. In addition, a small amount of water in which bloodworms had been macerated was tipped into the tank to introduce olfactory cues. Before each trial, groups of five fish (or in one case of four fish) were deprived of food for at least 12 h, placed into the settling compartment with the tunnel blocked and held for 5 min. The cover of the exit tube was then removed and the time (in s) taken for each fish to leave the settling chamber was recorded, up to a maximum of 15 min; fish that failed to emerge were given a notional, high score of 1000 s. Nineteen fish were tested in four groups, with 10 repeats of the screening procedure. All the fish were housed together between trials.

**Screening for competitive performance**

No direct aggression between *C. carpio* was observed; instead competition for food took the form of jostling for favourable feeding positions. To quantify effectiveness in such scramble competition, *C. carpio* were deprived of food for at least 12 h and placed in a glass tank ($32.5 \times 22.5 \times 22.5$ cm, filled to a depth of 15 cm with water at $c. 18^\circ$ C) in the same groups in which they were screened for risk taking (see above). A clear plastic tube (5 cm in diameter and 45 cm in length) was placed vertically in the centre of the tank, with one end in contact with the substratum and the other projecting above the water surface. The tube had a hole cut into it at the base, of a size (2.5 cm high and 2 cm wide) chosen so that it would accommodate the snout of just one fish at a time. The hole was orientated towards the front of the tank, so as to be clearly visible to an observer. Bloodworms were introduced into the tube by pipette; these were clearly visible to the fish, which tracked the larvae as they sank to the bottom of the tube and jostled for a feeding position at the hole, circling the base of the tube pushing for access to the feeding hole. The identity of the first three fish that took larvae was recorded and the tube was then removed, allowing all the fish to consume the remaining food. Nineteen fish were tested in four groups, with 10 repeats of the screening procedure. All the fish were housed together between trials. The median interval between tests was 5 days, with a maximum of 10 days. Cases in which no fish in the group emerged were deemed to be the result of uncontrolled disturbance and discarded from the analysis; in such cases, the fish were re-tested on the following day. All the fish were housed together between trials.

**Statistical analysis**

Preliminary examination of the data indicated that time of emergence from the settling chamber was negatively related to both water temperature and trial number. The distribution of emergence times was highly skewed, making it difficult to allow for these effects statistically. The response of individual fish to the potentially dangerous environment was therefore
quantified by the sequence in which the fish emerged; since fish were always tested in the same groups, any effects of temperature and trial were allowed for in this way. Individual differences in risk taking were examined using Kruskal–Wallis ANOVA on the sequence in which fish emerged from the settling chamber. Consistency of response was examined by the Kendall coefficient of concordance (Kendall’s $W$) for emergence sequence, within each group (Siegel & Castellan, 1988). Since significant consistency was identified, and the overall performance of individual fish in the risk-taking trials was summarized by the median of their emergence ranks across all tests. The relationship between $L_T$ and overall emergence score was examined using non-parametric correlation analysis.

In the scramble-competition tests, in most cases, at least three fish acquired food. A score for competitive performance in any given trial was derived by giving a score of 3 to the fish that ate first, 2 to the fish that ate second, 1 to the fish that ate third and 0 to all other fish. Individual differences in competitive performance were examined using Kruskal–Wallis ANOVA. Consistency of response was examined by the Kendall coefficient of concordance for feeding score within groups. Competitive performance over all tests was quantified by the number of times each fish obtained food compared with the number of opportunities that each had to feed. The relationship between $L_T$ and overall competition score and between overall risk-taking score and overall competitive score was examined by non-parametric correlation analysis.

**EXPERIMENT 2: METABOLIC RATE IN RISK-TAKING AND RISK-AVOIDING CYPRINUS CARPIO**

**Fish and husbandry**

*Cyprinus carpio* were artificially reproduced crossbreeds of known production lines at the Polish Academy of Sciences’ Institute of Ichthyobiology and Aquaculture, Zaborze, Poland, bred under natural conditions in earthen ponds. In September 2007, 1 year-old fish (mean $\pm$ s.e. mass = 25.3 $\pm$ 1.07g) were harvested, treated for infection and stocked in groups of c. 700 in 3000 l tanks with re-circulated water under natural light conditions (49.87$^\circ$N) before screening.

**Screening for coping strategy**

As in experiment 1, risk taking was assessed by screening the rate at which individuals explored an unfamiliar, potentially dangerous environment, again testing fish in groups since isolation proved extremely stressful. Since a large number of fish were to be screened (580 fish altogether) and it was important to keep handling to a minimum, the system for estimating risk taking was modified to allow risk-taking phenotype to be assessed in unmarked fish.

After settling for 1 week, *C. carpio* were deprived of food for at least 12 h and 10 randomly selected fish were hand-netted from their holding tank into buckets, covered and allowed to settle for a further 15 min before being tipped gently into a settling area at one end of a well-lit tank (1.5 by 1 by 1 m). The settling area comprised a covered circular opaque black compartment (diameter 50 cm) fitted at the base with a closable exit tube (diameter 7.5 cm). A covered area at the opposite end of the main tank was connected by a closable gate into the fish collection area. The fish were allowed to settle for 5 min, during which feed extract (prepared by soaking food pellets in water) was gently tipped into the test compartment just in front of the exit tube. The cover of the exit tube was then removed and a two-phase observation period initiated. The first phase ended after the first three *C. carpio* had emerged from the settling area or after a period of 10 min if fewer than three fish emerged during this time. The exit tube was then closed and the fish that had emerged gently edged into the fish collection compartment and the gate closed. These fish were classified as risk takers. A second small amount of feed extract was added in front of the exit tube, which was then opened and a second recording period started, during which a further four fish were allowed to emerge and the exit tube was closed again. These fish were classified as of an intermediate risk-taking phenotype. The three remaining fish were confined in the shelter by replacing the lid; these fish were classified as risk avoiders. If fewer than four intermediate fish emerged during 15 min of observation, all the remaining fish were classified as risk avoiders. After
screening, the intermediate fish were discarded and risk-taking and risk-avoiding fish were housed in separate 250 l holding tanks in a closed circulatory system and maintained at a temperature of 20° C, range ± 0.5° C.

**Measuring resting metabolic rate**

Resting metabolic rate measurements were carried out using a peristaltic multi-channel pump (MLW Labortechnik, Ilmenau, Germany; Model DP 2.2). Six silicone tubes (4 mm internal diameter, 6 mm external diameter and 120 cm length, Deltalab S.L.; www.deltalab.es) supplied 95%, range ± 1% saturated water (oxygen concentration 8.8–9 mg l⁻¹, water temperature 20.0° C, range ± 0.5° C) from a reservoir tank (88 l) to the pump. A 15 cm section of tubing (diameter 5 mm) fed water through the pump before connecting to smaller diameter tubing (4 mm by 120 cm length) that supplied water to each of six, 65 l airtight respirometer chambers. Flow rate was measured before each trial and was equalized between containers by tightening or loosening a clamp on the peristaltic pump (mean flow rate = 0.88 ml s⁻¹, minimum = 0.83 and maximum = 0.97 ml s⁻¹). Respirometer chambers were located in a section [120 cm (length) by 60 cm (height) by 40 cm (width)] of a trough (500 × 60 × 40 cm) and bathed in 3 cm of temperature-controlled running water. Chambers were placed in a row and the sides blacked-out to prevent visual contact between fish. Oxygen concentration was continually measured using six oxygen probes (model WTW Oxi Cellos 320; www.wtw.com) and an oxygen meter (WTW Multiline P3). Before the start of a trial, the aquarium section containing the respirometer chambers was covered with black material to block out light. At the end of the trial, oxygen probes were cleaned and calibrated for the start of a new trial and chambers and connecting tubing were flushed with tap water to remove any organic matter.

For each screening, six fish classified as risk takers and six as risk avoiders were hand-netted from their respective holding tanks and transferred in buckets to the experimental room. Fish were weighed to the nearest 1 g and measured to the nearest 0.1 cm before being paired by mass for the trial. Fish were tested in pairs of the same risk-taking phenotype, since isolated fish failed to settle. Pairs of fish were transferred by hand to the respirometer chamber, which was closed underwater to prevent air bubbles entering the system. After all the chambers were placed in the trough and covered with a blackout cloth, the first reading of oxygen concentration was noted. Thereafter, oxygen concentration was measured every 15 min over a period of 4 h; fish took an average 3 h to acclimate to the experimental setup. On the following morning, between 0600 and 0615 hours, c. 12 h after the last oxygen reading had been taken, a final reading was noted. This figure was considered to reflect the resting level of oxygen consumption of inactive and unfed pairs of fish; rate of oxygen uptake was calculated as mg O₂ kg⁻¹ h⁻¹. Altogether, 21 pairs of fish from each risk-taking category were screened in this manner. The mean mass of the fish used was 25.32 g.

**Statistical analysis**

Body mass and mass-specific resting metabolic rate in *C. carpio* classified as risk takers and risk avoiders were compared using *t*-tests. There was insufficient overlap in size between the two categories of fish for any independent effect of body size on mass-specific resting metabolic rate to be examined statistically. Two empirically derived equations for the relationship between body mass and resting metabolic rate in *C. carpio* (Yamamoto, 1991; Guan et al., 2008) were therefore used to calculate the expected difference in mass-specific resting metabolic mass between the two groups of fish based on size alone.

**Experiment 3: Stress Responsiveness in Risk-taking and Risk-Avoiding Cyprinus carpio**

*Cyprinus carpio* were artificially reproduced crossesbreeds of known production lines at the Polish Academy of Sciences’ Institute of Ichthyobiology and Aquaculture, Zaborze, Poland, bred under natural conditions in earthen ponds. In April 2006, 1 year-old fish (mean ± s.e. mass: 64.7 ± 2.4 g) were harvested, treated for infection and stocked in groups of 70 in 60 l tanks with re-circulated water under natural light conditions (49.87° N) before screening.
Screening and sampling

_Cyprinus carpio_ were screened for coping strategy in groups of 10, as described for experiment 2, using the sequence in which individuals emerged from a safe compartment to explore an unfamiliar, potentially dangerous environment. Intermediate fish were discarded, and fish classified as risk takers and risk avoiders were given batch marks and held in groups of 20 (10 risk takers and 10 risk avoiders) in 60 l tanks under normal aquarium conditions (7–8 mg O₂ l⁻¹ and 20° C) and fed daily with 2 mm diameter pellets (Aller Aqua; www.aller-aqua.com) at a ration of 2% fish biomass per day. After 10 weeks, six risk-taking and six risk-avoiding fish were injected with phosphate-buffered saline (this was used as a control for an inflammatory challenge with bacterial lipopolysaccharide) 24 h before being killed. Immediately after killing, blood samples were collected and assayed for plasma concentrations of lactate (Lactate Dry-Fast, Sentinel Diagnostics Sentinel CH SpA; www.sentinel.it) and glucose (HYDREX colorimetric end-point enzymatic assay; www.hydrex.pl). The brain and head kidney of the same fish were removed, frozen in liquid nitrogen and transported on dry ice to the Department of Animal Physiology of the Autonomous University of Barcelona, Spain.

Measuring cortisol receptor expression

Quantitative polymerase chain reaction (PCR) was used to measure gene expression (Acerete et al., 2007). Four micrograms of total RNA was taken from pooled brain and head kidney samples (n = 6) to synthesize cDNA with SuperScript III RNase transcriptase (Invitrogen; www.invitrogen.com) and oligo-dT primer (Promega; www.promega.com). cDNA was diluted 1:100 for the amplification of selected genes and 1:1000 for 18S and used as a template with primers designed for Q-PCR. 18S forward primer; 5'-CGA GCA ATA ACA GGT CTG TG-3', reverse primer; 5'-GGG CAG GGA CTT AAT CAA-3', amplicon size obtained 212 bp. Cortisol receptor, forward primer; 5'-CCA GCA AGA ACT GGC AAC GA-3', reverse primer; 5'-TGA TGA TCT CCG CCA GCA TT-3', amplicon size 150 bp. Wells (20 μl final volume) contained 10 μl of iQ SYBR Green Supermix (Bio-Rad; www.bio-rad.com), 500 nM concentration of forward and reverse primers and 5 μl of cDNA. Controls lacking cDNA and controls containing RNA were included. Reactions were run in a MyiQ thermocycler (Bio-Rad) under the following protocol: 5 min initial denaturation at 95° C, followed by 40 cycles of 10 s denaturation at 95° C and 30 s at annealing temperatures (both primer sets 60° C) and a final melting curve of 81 cycles (from 55 to 95° C). All samples were run in triplicate, and fluorescence was measured at the end of every extension step. Threshold cycle (Cₜ) values for each sample were expressed as n-fold differences, calculated relative to control and normalized for cortisol receptor (CR) against those obtained for 18S. Transcripts were sequenced to ensure amplification was specific: products were visualized under UV light in a 1% agarose gel containing 1 mg ml⁻¹ ethidium bromide, purified using MiliElutegel purification system (Qiagen; www.qiagen.com), cloned into PGEM-T Easy Vector (Promega) by terminal transferase activity (TA) cloning and transfected into competent _Escherichia coli_ IM 109 cells (Promega). Plasmid DNA was isolated by Nucleospin Quickpure (Marcherey Nagel; www.mn-net.com), digested with EcoRI (Promega) and sequenced with T7 primer.

Statistical analysis

Body mass and lactate and glucose concentrations in _C. carpio_ classified as risk takers and risk avoiders were compared by _t_-tests.

RESULTS

EXPERIMENT 1: A BEHAVIOURAL SYNDROME IN _CYPRINUS CARPIO_?

Screening for risk taking showed considerable variability in emergence time over the whole dataset, with the fastest fish to emerge taking 43 s and the slowest of
those that emerged at all taking 843 s. There was a highly significant individual fish effect on emergence rank across trials [Fig. 1(a); Kruskal–Wallis $H = 58.75$, d.f. = 18, $P < 0.001$]. In addition, emergence rank was significantly concordant across trials for individual fish in all four groups (Kendall’s $W = 0.45$, $P < 0.05$, 0.79, $P < 0.01$, 0.55, $P < 0.01$ and 0.74, $P < 0.01$). There was no relationship between $L_T$ and median emergence time for individual fish over all tests (Spearman’s rho, $r_s = -0.24$, $P > 0.05$).

In the trials for competitive performance, within each group, certain fish were consistently among the first to feed, while others rarely gained access to the feeding point. There was a highly significant individual fish effect on feed score across trials (Kruskal–Wallis $H = 50.39$, d.f. = 18, $P < 0.001$, adjusted for ties). In addition, feeding score was significantly concordant across trials for individual fish in all four groups (Kendall’s $W = 0.54$, $P < 0.05$, 0.73, $P < 0.01$, 0.65, $P < 0.01$ and 0.55, $P < 0.05$). All fish fed actively once the tube had been removed. Overall competitive
ability, measured by the proportion of potential feeding opportunities at which each fish gained food, ranged from 1.0 to 0.2. There was no relationship between $L_T$ and overall competitive ability ($r_s = 0.04$, $P > 0.05$). There was a noisy but significant positive relationship between risk taking, assessed by median emergence rank, and competitive ability, assessed by the proportion of potential feeding opportunities at which each fish gained food [Fig. 1(b); $r_s = 0.57$, $P = 0.01$].

**EXPERIMENT 2: METABOLIC RATE IN RISK-TAKING AND RISK-AVOIDING *CYPRINUS CARPIO***

Mean ± s.e. masses were 23.0 ± 0.9 and 27.7 ± 1.8 g for risk takers and risk avoiders, respectively ($t = 3.53$, d.f. = 33, $P = 0.001$). The mean ± s.e. mass-specific resting metabolic rates for risk-taking and risk-avoiding fish are shown in Fig. 2. Values were variable within risk-taking phenotypes, but there was a significant difference between the two groups, with risk takers having on average a higher mass-specific resting metabolic rate than risk avoiders ($t = 2.33$, d.f. = 35, $P < 0.05$).

**EXPERIMENT 3: STRESS RESPONSIVENESS IN RISK-TAKING AND RISK-AVOIDING *CYPRINUS CARPIO***

Mean ± s.e. masses were 66.4 ± 3.9 and 63.0 ± 2.9 g for risk takers and risk avoiders, respectively ($t = 0.7$, d.f. = 29, $P > 0.05$). The mean ± s.e. plasma concentrations of glucose and lactate in *C. carpio* classified behaviourally as risk takers and risk avoiders are shown in Fig. 3. Levels of both were significantly higher in risk-avoiding than in risk-taking fish (glucose: $t = 3.6$, d.f. = 29, $P = 0.001$; lactate: $t = 7.03$, d.f. = 33, $P < 0.001$). The levels of expression of CR genes in the brain and head kidney of *C. carpio* classified as risk takers and risk avoiders
following a stressor (control injection with phosphate-buffered saline) are shown in Fig. 4. These values are from pooled tissue from six fish of each risk-taking category, the error bars referring to technical replicates. In both cases, CR expression levels were markedly higher in risk-avoiding fish than in risk takers, being at least two-fold higher in the brain and some four-fold higher in the head kidney.

**DISCUSSION**

This study has shown that marked differences exist in the behaviour of *C. carpio*, both when exploring an unfamiliar and potentially dangerous environment and when forced to jostle for position at a spatially restricted feeding site. The fish showed significant consistency both in response to a novel, potentially dangerous environment (risk taking) and in ability to monopolize a restricted feeding site (competitive ability). In addition, there was a significant positive relationship between individual performance in these two contexts, with fish that explored more quickly in the novel environment being more likely to gain preferential access to a restricted feeding
Brain

Risk avoiders
Risk takers
Risk avoiders
Risk takers

Brain

Head kidney

CR expression

0
0.5
1
1.5
2

Fig. 4. Mean ± s.e. corrected cortisol receptor (CR) expression in Cyprinus carpio classified as risk takers and risk avoiders on the basis of time taken to explore a novel environment.

location. Individual variability in risk taking has been reported for other species of fishes that are from schools or shoals, albeit with a negative relationship between risk taking and tendency to form schools (Wilson et al., 1993; Budaev, 1997).

Two features of the methods used for screening risk taking in C. carpio were made necessary by the species-specific behaviour of these fish. In the first place, as indicated above, fish placed in isolation become highly disturbed; this was deemed likely to obscure any underlying differences in response to risk, had they been screened singly. Recording patterns of emergence in C. carpio held in small groups allowed such differences to be expressed and measured. This inevitability raises the possibility that individual behaviour (and indeed the relationship between behavioural traits; Van Oers et al., 2005) may have been affected by social facilitation or reduced perceived risk. Thus, G. aculeatus in small groups resume foraging more quickly after a simulated predatory attack than do isolated fish (Webster et al., 2007) and choice among shelters is strongly influenced by the behaviour of shoal mates (Ward et al., 2008). In perch Perca fluviatilis L., the behaviour of fish in small groups is influenced by that of other group members, although inherent individual differences in response to novelty and risk are still apparent (Magnhagen & Staffan, 2005). Scrutiny of emergence times in the data from the present tests showed that there were a number of occasions on which more than one fish emerged within a short space of time, suggesting that some were indeed following others. Even so, since the emergence tube was designed to let just one fish pass at a time, the sequence of emergence remains to some extent the result of individual choices and using emergence rank rather than emergence time in these tests takes account of this.

A second feature of the risk-taking test was that, for reasons given, the fish could see and smell food in the novel environment, so the test could potentially have been measuring differences in feeding motivation rather than differences in risk taking. Certainly, differences in food deprivation can influence the risks that prey animals take in a variety of situations (Laland & Reader, 1999; Petrie & Ryer, 2006; Fraker, 2008; McCormick & Larson, 2008), the reason why the fish in this study were
kept on a standard feeding regime, being fed to satiation for several weeks before screening and were deprived of food for 12 h before each trial. Most studies of variable risk taking in fish have been carried out on juvenile animals in which the main reason for exploring a novel environment is ultimately to find food. A trade-off between behaviour related to foraging and risk of predation is therefore implicit in most novel environment tests and the use of food cues in the present study simply makes this explicit.

Thus, it seems that in *C. carpio* a behavioural syndrome exists, in the sense that two individually consistent traits (risk taking and competitive ability) are correlated at the individual level. This is in agreement with studies on a number of vertebrate species, including some fishes (Sih *et al.*, 2004). The results presented here also suggest that in *C. carpio* the risk-taking behavioural phenotype is associated with a relatively high mass-specific resting metabolic rate, while the risk-avoiding phenotype is associated with a lower rate. One possible complication here is that the fish classified as risk takers on the basis of their behaviour were slightly but significantly lighter than those classified as risk avoiders. Since it is a common finding that mass-specific resting metabolic rate falls with body mass in fishes (Clarke & Johnston, 1999), including *C. carpio* (Oikawa & Itazawa, 1984), the observed difference could potentially be the result of the difference in body size rather than of the difference in risk taking. Because the difference in mass though statistically significant is very small, however, two separate empirically derived equations for *C. carpio* (Yamamoto, 1991; Guan *et al.*, 2008) both predict no difference in mass-specific resting metabolic between the groups based on size alone. It is therefore concluded that the observed difference in resting metabolic rate (c. 17%) is indeed the result of the difference in risk-taking phenotype. This fits the ‘performance model’ described by Careau *et al.* (2008), whereby an active, aggressive life style is associated with well-developed machinery for acquiring and processing food and hence with higher than average maintenance costs.

As predicted by a growth-mortality trade-off (Stamps, 2007), several studies using fishes have found bold, risk-taking individuals to be heavier than more timid individuals from the same population (Biro & Stamps, 2008), but the situation is complex because other studies have found the opposite. For example, in *Brachyrhaphis epischiopi* (Steindachner), for any given body length, fish that are fast to emerge from shelter are heavier than slow emergers (Brown *et al.*, 2007). In the same species, however, shorter fish tend to emerge from shelter sooner, perhaps because their immediate nutritional needs are greater (Brown & Braithwaite, 2004). Yet, other studies have found no relationship between body size or condition and risk-taking behaviour (Budaev, 1997). The fact that risk taking was associated with larger body size in just one of the present groups further highlights the complexity of the relationship between behaviour, body size and nutritional status.

The present results also suggest that *C. carpio* classified behaviourally as risk takers show low responsiveness when stressed, indicated both by lower plasma lactate and glucose levels and by lower expression of CR genes in the brain and head kidney. This is consistent with the published data on *O. mykiss*, in which behavioural and physiological differences between lines selected for stress responsiveness (Øverli *et al.*, 2005) fit the pattern described for proactive and reactive animals, as seen in mice and rats (Korte *et al.*, 2005; Koolhaas *et al.*, 2007).
By documenting consistent and correlated individual differences in risk taking and aggression and by relating these to differences in stress physiology, the present study provides another example of coping strategies in fishes. By (tentatively) demonstrating higher resting metabolic rates in proactive individuals, it also contributes to the developing literature on the relationship between energy metabolism and individual behavioural profiles and on the selective pressures that maintain such diversity within populations. The existence of a behavioural syndrome or coping strategies in C. carpio has a number of practical implications. This species is an important species for aquaculture with a long history of domestication (Balon, 1995), and the evidence suggests that domesticated strains are generally less responsive to stressors (Hancz et al., 2000; Tanck et al., 2002). Even so, a number of husbandry practices have been shown to induce stress responses in C. carpio, including handling (Saeij et al., 2003), confinement and crowding (Ruane et al., 2002), transport (Dobsikova et al., 2006) and harvest and post-harvest storage (Svobodova et al., 2006). Given the documented inherited variability in stress responsiveness in C. carpio (Tanck et al., 2001, 2002), selective breeding for fish with low stress responsiveness might well generate fish with some of the characteristics that promote welfare in aquaculture (e.g. low response to stressors). If behavioural and physiological variability is indeed organized into coping strategies in C. carpio, it might be possible to use behaviour as a proxy for physiology in mass screening. On the other hand, the existence of coping strategies could mean that selection for low stress responsiveness would generate more intense competition for food (Huntingford & Adams, 2005). Where fishes are farmed for release (either for reintroduction of endangered species or for restocking of depleted fisheries), the existence of coping strategies means that it is important to develop husbandry systems that ensure a range of coping styles among released fishes (Huntingford, 2004).

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