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A Comparative Analysis of the Preference for Dark Environments in Five Teleosts

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The present article tried to establish dark/light preference in five different species of teleosts. We proposed, using the data obtained with this method in zebrafishes (*Danio rerio*), Cardinal-tetras (*Paracheirodon axelrodi*), lambaris (*Astyanax altiparanae*), Nile tilapias (*Oreochromis niloticus*), guppies (*Poecilia reticulata*) and banded-knife fishes (*Gymnotus carapo*), that preference for dark environments is a reliable and low-cost index of anxiety/fear in those species. A scototactic pattern of exploration was found in all species, and the pattern of locomotion in the white environment suggests its aversiveness for those species, with the exception of *G. carapo* and *O. niloticus*. A comparative analysis uncovered species differences in approach-avoidance dimensions of the task. The data are discussed in terms of the behavioral ecology of the animals and prey-predator relationships, suggesting a link with predator defense strategies in teleost.

The dark/light preference model is already established as an "ethoexperimental" anxiety model in rodents (cf. Bourin & Hascöet, 2003). It is based on the natural aversive quality of brightly-lit environments for mice, shaping

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a conflict situation in which the animal must deal with its natural tendency to explore in face of the aversiveness of the environment. The rodent dark/light preference model is an *exploration* model, in the sense that it measures locomotor activity in both environments as an index of anxiety (Green & Hodges 1991; Prut & Belzung 2001; Belzung & Griebel 2003; Hascöet, Bourin, & Dhonnchadha, 2001); there are other, non-locomotor, models of anxiety (eg., inhibitory avoidance), but those are not of concern for the objectives in this article. Locomotor models of anxiety use exploratory behavior (defined as “a species-specific behaviour pattern concerned with the gathering of information” concerning the environment: O'Keefe & Nadel, 1978, p. 242) as an index of anxiety or anxiety-like states, relating it to foraging behavior or to appraisal of novel environmental stimuli (Belzung & Griebel 2001; File, 2001). The main rationale is that exploratory behavior would correlate with neophobia, a tendency to avoid new environments (Misslin & Cigrang 1986), forming a mixed pattern of behavior that consists in gradual approaching and exploration of the new environment associated with "scanning" and "risk-assessment" behaviors.

Ethoexperimental models use variables that are akin to the concept of “antipredator apprehension” from behavioral ecology (risk assessment, defensive distance, predatory imminence continuum, risk associated suppression of competing motivational systems; Kavaliers & Choleris, 2001). Apprehension is considered to reflect a motivational state, and is defined as “any reduction in attention to other activities (e. g., foraging, mate seeking) as a result of increasing the allocation of attention to detecting and/or responding to potential predators” (Kavaliers & Choleris, 2001, p. 579). Exploratory apprehensive behavior (denoting the pattern of exploratory behavior in such situations), in naturalistic situations as well as in locomotor-based anxiety models, is a compromise between predator avoidance and the benefit of an alternative activity (Ydenberg & Dill, 1986). Blanchard and Blanchard (1988) proposed the concept of “defensive distance”, analogous to the “antipredator apprehension” delineated in behavioral ecology. Defensive distance is a “statistical appraisal” of sorts that defines the probability of threat; it is a dimension controlling the type of defensive behavior observed (explosive attack, freezing, flight, risk assessment; Blanchard & Blanchard, 1990). Apprehension is understood as a continuum, and is defined as “any reduction in attention to other activities (...) as a result of increasing the allocation of attention to detecting and/or responding to potential predators” (Kavaliers & Choleris, 2001, p. 579); various levels of apprehension “lead prey to select a certain optimal level of vigilance, that is staying alert (i.e., scanning behavior, head up) so as to detect an approaching enemy, in response to their perceptions of a predator's whereabouts” (Kavaliers & Choleris, 2001, p. 579). Wilson, Clark, Coleman, & Dearstyne (1994) defined a “shyness-boldness” continuum which they based on a 'propensity to take risks', which is analogous to individual differences in antipredator behavior. Thus, in a particular situation that would require antipredator behavior, and individual that performs more risky behaviors is considered bold, whereas one which avoids risk is called shy.

In the beginning of the 1980s, Gray (1982) related the O’Keefe & Nadel (1978) model of exploratory behavior to possible anxiety-generating effects of the

exploration models. The rationale in Gray's analyses is the existence of a putative *behavioral inhibition system* that detects a conflict between two motivations – in the mentioned cases, between neophobia and a motivation to explore – and switches behavioral programs in response to this conflict. As such, the behavioral inhibition system computes trade-offs between both approach and avoidance motivations, and switches behavior in accordance to the result of this computation. There have been some suggestions that this system involves multiple structures, including (in mammals) the periaqueductal gray, the medial hypothalamus, the amygdala, the septo-hippocampal system, and the cingulate and prefrontal cortices (McNaughton & Corr, 2004). The behavioral inhibition system is involved in what is called "fear" and "anxiety", both innate and conditioned (Antoniadis & McDonald, 2001; Misslin, 2003; Rosen, 2004); indeed, fear has been defined as "a functional defense behavior system representing a part of the innate species-specific behavioral repertoire (ethogram), basic to the survival of individuals and species" (Misslin, 2003, p. 55).

The rodent dark/light preference model has been pharmacologically validated, and is sensitive to many parametric manipulations (Hascoët et al., 2001). One given manipulation is considered to have an antianxiety-like effect – ie, it has an effect similar to that of classic antianxiety drugs – if it facilitates exploratory activity, and this effect is dependent on the baseline level of the control group. The main variable analyzed is the number of transitions between the two compartments of the apparatus; it is indexical of activity/exploration, while the habituation over time and the time spent in each compartment reflects the aversiveness of the environment. The model is based on spontaneous activity, hence not requiring prior training of a response. The main advantages are ease of use and velocity of data production.

The proposed actinopterygian dark/light preference task is a modification of an experimental manipulation used in the 1970s to establish the effects of noradrenergic substances on the scotophobic (ie, dark-avoiding) behavior of pinealectomized or scotophobin-injected goldfish (Satake & Morton, 1979). Recently, the proposed model was used to establish dark/light preference in the zebrafish *Danio rerio* (Serra, Medalha, & Matiolli, 1999), the bluegill *Lepomis macrochirus*, the crucian carp *Carassius langsdorfii* (Yoshida, Nagamine, & Uematsu, 2005), the goldfish *Carassius auratus* (Gouveia Jr et al., 2005; Yoshida et al., 2005), and in the poeciliid *Brachyraphis episcopi* (Brown, Jones, & Braithwaite, 2005), and to screen for the neurobehavioral effects of methylmercury (Gouveia Jr. et al., unpublished) and ethanol (Gerlai, Lahav, Guo, Rosenthal, 2000) on the zebrafish. The main advantage of this task is the presentation of a clear conflict situation for the fish; however, most models that investigated innate "fear"- and "anxiety"-like behavior in fishes did not use such conflict. With the exception of predator inspection tests (eg., Budaev, 1997a; Bleakley, Martell, & Brodie III, 2006; McCartt, Lynch Jr., & Johnson, 1997), most innate anxiety tests use the exploration of an open field to measure this variable (Crawshaw, 1975; Gervai & Csányi, 1985; Kleerekoper et al., 1970; Mikheev & Andreev, 1993; Mok & Munro, 1998; Warren & Callaghan, 1976), and aim to describe individual variability in "shyness-boldness" continua (Brown & Braithwaite, 2004; Brown et

al., 2005; Budaev, 1997b; Moretz, Martins, & Robison, 2007; Ward, Thomas, Hart, & Krause, 2004; Wilson, Coleman, Clark, & Biederman, 1993). This "shyness-boldness continuum" can be mapped to Budaev's (1997a, 1998) two dimensions of "temperament" in fishes (Activity-exploration and Fear-avoidance), which, in its turn, are trait instantiations of approach-avoidance state dimensions (Craig, 1917; McNaughton & Corr, 2004). *Ex hypothesi*, these dimensions are best analysable using conflict models.

The present article analyses dark/light preference as a reliable and low-cost ethoexperimental model of exploratory behavior and anxiety-like reactions in some species of teleost fish. We propose that scototaxis (preference for dark environments) can be used to assess stress, fear and anxiety in a wide array of fish species that present similar feeding ecology. We report the data obtained with this method in zebrafishes (*Danio rerio*); Cardinal-tetras (*Paracheirodon axelrodi*) and lambaris (*Astyanax altiparanae*); Nile tilapias (*Oreochromis niloticus*); and the banded-knife fish (*Gymnotus carapo*) (all references for taxonomy were taken from Helfman, Collette, & Facey, 1997). These species all present dark-colored backs; Table 1 also presents further information on ecogeographical and ecological contexts. These species were chosen for particular reasons: zebrafish is a "model animal" in embryology and genetics; characid fishes are very common neotropical species; Nile tilapias are commercially explored animals; guppies are common subjects in behavioral ecology; and *G. carapo* is a weakly electric fish, and analysing its preference for an environment could also shed some light on its visual status. Also, the phylogenetic relations between those species are well-resolved (Helfman et al., 1997). In all experiments, methodology was the same (as described in "General methods", below).

Table 1

Ecological and environmental profiles of the species chosen, with focus on ecogeography and turbidity/depth of the environment. Data was taken from FISHBASE (http://www.fishbase.org). Refer to text for more information.

Family	Species	Environment	Climate	Ecogeography
Cyprinidae	*Danio rerio*	Benthopelagic	Tropical	Inhabits streams, canals, ditches, ponds and beels
Characidae	*Paracheirodon axelrodi*	Pelagic	Tropical	Inhabits middle water layers
Characidae	*Astyanax altiparanae*	Benthopelagic	Tropical	Inhabits streams, canals, ditches, ponds and beels
Gymnotidae	*Gymnotus carapo*	Benthopelagic	Subtropical	Inhabits turbid slow moving or standing waters
Poeciliidae	*Poecilia reticulata*	Benthopelagic	Tropical	Inhabits slow-flowing or still water near the margin of pools among vegetation.
Cichlidae	*Oreochromis niloticus*	Benthopelagic	Tropical	Inhabits the littoral zone of lakes, but was introduced in other environments as well.

Methods

Equipment

Three acrylic aquaria of equal measures (15x10x45 cm), with diverse colors according to the treatment (white (WW), black (BB), or half black/half white (BW); walls and bottom colored), with the water column kept to 10 cm. The colored material chosen was not reflective, in order to avoid the tendency of those animals which present shoalling and/or schooling tendencies to behave in relation to their own reflection. All the test aquaria contained sliding central doors, colored with the same color of the aquarium side, thereby defining a central compartment with 15x10x10 cm. For the banded-knife fish, aquaria dimensions were different. Since those animals measured 10.0±2.1 cm at the time of testing, their test aquaria measured 15x10x55 cm (with the central compartment measuring 15x10x20 cm). During experiments, each aquaria was rotated after each trial, so as to eliminate orientation effects. Aquaria were illuminated by environmental light (60W light bulb, located at 1.80 m above the aquarium top) which kept illumination uniform and constant between trials. The aquaria are shown in Figure 1.

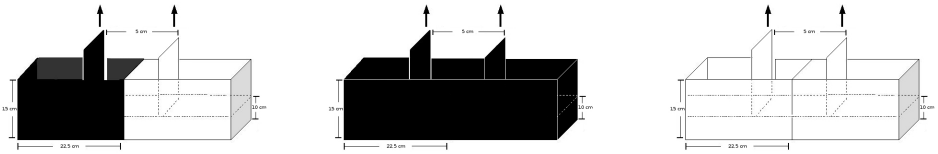

Figure 1. Schemata of the three test aquaria.

Animal rearing

Animals were acquired in a local pet shop (zebrafish, Cardinal-tetra, guppy), in a local fisheries (lambari, banded-knifefishes), or at the fisheries in the hatchery at UNESP/São José do Rio Preto, and kept in the laboratory for at least two weeks before the experiments. All subjects were kept in collective maintenance aquaria (60x25x40 cm), with one tank per species; the water was reconstituted and buffered (Mydor Target 7.0 buffer), and the animals were acclimated for at least 7 days, with constant filtering, temperature control (27±2 ºC), lighting (12/12 h, beginning of the cycle at 0700 pm) and feeding (Oscar Gold pellet ration). To prevent intervening motivations, all animals were fed once a day, and not fed in the day the experiment took place. Animals were not used for any other experiment besides those presented in this paper. Rearing and welfare conditions were in accordance with the standards set by the ASAB/ABS (2006) and COBEA/Brazil, and were approved by the Institution's Ethics Committee.

Zebrafish. 51 adult zebrafishes, of undetermined sex, were used in the experiment. Animals were acquired in a local pet shop (*AquaMundi*, Bauru/SP, Brazil), and measured 2.63±0.09 cm at the time of the experiment.

Cardinal-tetra. 27 adult Cardinal-tetras, of undetermined sex, were used in the experiment. Animals were acquired in a local pet shop (*AquaMundi*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Subjects measured 2.45±1.0 cm at the time of the experiment.

Banded-knife fish. 24 adult banded-knife fishes, of undetermined sex, were used in this experiment. Animals were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Subjects measured 10.0±2.1 cm at the time of the experiment.

Lambaris. 24 adult lambaris, of undetermined sex, were used in this experiment. Subjects were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Animals measured 5.5±0.8 cm at the time of the experiment.

Nile tilapias. 60 adult Nile tilapias (30 male, 30 female), reared at the hatchery in UNESP/São José do Rio Preto, were used in this experiment. Male tilapias measured 5.48±0.65 cm,

and females measured 5.77±0.61 cm, at the time of the experiment.

Guppies. 54 adult guppies (27 male, 27 female) were used in this experiment.

Subjects were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described in the "General methods" section. Female guppies measured 2.81±0.64 cm and male guppies measured 3.07±0.31 cm at the time of the experiment.

Procedure

All experiments used the same procedure. Animals were randomly divided between the black/white, white/white and black/black treatments, measured, then subjected individually to a single observation session; each treatment was tested in a different aquarium, and animals were used for a single treatment. To avoid effects of repeated exposure to the apparatus, only a single session was run with each animal, and no replicates were made. Thus, the data collected refers to one session in each aquarium per species. The animals were placed in the central compartment for five minutes (habituation), after which the sliding doors were removed. The animals were then allowed to freely explore the aquarium. The session is terminated after 900 s. Total time in each environment, number of midline crossings, permanence time in each environment (total time/midline crossings), and latency for the first choice of compartment were recorded as variables. The first and third variables are measures of preference (Noakes & Baylis, 1990), while the second and fourth variables represent locomotor behavior (cf. Warren & Callaghan, 1976). It is hypothesised that preference variables are going to be affected by the type of aquaria, being significant only in the black/white aquarium, while locomotor variables will be greater in the white/white aquarium. As such, if the white environment is indeed aversive, locomotion will be heightened in the white/white aquarium, and animals will take refuge in the black environment of the black/white aquarium. Even though the confinement in the central compartment for habituation allowed for contact with both sides of the aquarium, data from those animals that did not cross the midline in the 900 s session were discarded, to prevent false positives in preference measures (Noakes & Baylis, 1990).

Statistical analyses

Since normality and equal variances were not assumed, non-parametric statistics were used in all analyses. Preference variables (total time and permanence time in either compartment of an aquarium) were analysed with Wilcoxon's signed rank tests. Motor variables (latency for first choice of compartment and number of midline crossings) were analyzed with one-way Kruskal-Wallis ANOVAs on Ranks, with aquarium as between-subjects factor, using Dunn's post-hoc tests whenever appropriate. All *P*-values were set at 0.05. To assess species differences, two independent variables (ratio between total time in the black and the white compartments of the black/white aquarium [B:W]; and number of midline crossings in the white/white aquarium [AltW]) were analysed with one way Kruskal-Wallis ANOVAs on ranks. The same variables were used in the assessment of "shyness-boldness", which was done using median rank values for the variables in each species. B:W was considered a proxy for preference for either environment, and AltW was considered a proxy for the aversiveness of the white environment. The data was analyzed using SigmaStat 3.1 (Systat Software, 2004).

Phylogenetic analysis

To control for phylogenetic dependence effects (Blomberg & Garland, 2002), a test for phylogenetic signal was made using the PHYSIG procedure (Blomberg, Garland, & Ives, 2003). The PHYSIG procedure tests for phylogenetic signal by randomization test, computing a test statistic *K* based on a phylogenetically correct mean and mean-squared errors of the data (calculated using the variance-covariance matrix derived from the candidate tree). One traitt (ratio between total time in the black compartment and total time in the white compartment of the black/white aquarium [B:W]) was analysed. Trait values were corrected for body size by computing a regression slope using phylogenetically independent contrasts (Felsenstein, 1985); the corrected value is the $\log_{10}$ of the ratio between the original trait value and body size raised to the IC slope. Phylogenetically independent contrasts regression was made using the PDTREE.EXE module of the PDAP package (Garland et al., 1993). Branch lengths were calculated using the Phylip GENDIST, with sequences

for cytochrome B mitochondrial DNA as distance parameters; sequences for cytochrome B were fetched from GENBANK. An Ornstein-Uhlenbeck model of trait evolution was assumed, and branch lengths were subsequently transformed by multiplying them by a value of d = 1.005. Transformed branch lengths were processed by the PDDIST.EXE module from the PDAP package to generate the variance-covariance matrix. After correction for body size effects and variance-covariance matrix determination, both data sets were parsed through the PHYSIG.M module of the PHYSIG package in order to determine phylogenetic signal. This should allow for selection between regular ANOVAs or Phylogenetic ANCOVAs for comparative data analysis (Garland et al., 1993).

Results

Table 2 presents the results, across aquaria, between species for the variables analyzed.

Latency for first choice and number of midline crossings

For *D. rerio*, statistical analysis showed a significant difference of latency time for the first choice of compartment (H[df = 2] = 9.63, p = 0.01) as a function of the aquarium used for test, with smaller values in the black/black aquarium in comparison to the white/white aquarium. There was no statistical difference in the latency for first choice (H[df = 2] = 3.24, p = 0.20) for *P. axelrodi*, *G. carapo* (H[df = 2] = 4.46, p = 0.11), *A. altiparanae* (H[df = 2] = 3.14, p = 0.21), female *O. niloticus* (H[df = 2] = 1.24, p = 0.54) or female *P. reticulata* (H[df = 2] = 2.20, p = 0.33). This same variable was significantly smaller in the black/white aquarium in male *O. niloticus* (H[df = 2] = 20.32, $p < 0.01$) and male *P. reticulata* (H[df = 2] = 8.56, p = 0.01).

The number of midline crossings was not statistically different in any of the aquaria for *D. rerio* (H[df = 2] = 3.847, p = 0.15), male *O. niloticus* (H[df = 2] = 1.81, p = 0.41) and male (H[df = 2] = 5.02, p = 0.08) and female P. reticulata (H[df = 2] = 4.76, p = 0.09). The white/white aquarium produced more midline crossings in *P. axelrodi* (H[df = 2] = 10.42, p = 0.01), *G. carapo* (H[df = 2] = 10.67, p = 0.01) and *A. altiparanae* (H[df = 2] = 15.52, $p < 0.01$), while the black/black aquarium produced more locomotion in female *O. niloticus* (H[df = 2] = 14.22, p = 0.01).

Total time and permanence time in each environment

In *D. rerio*, there was no effect of aquarium in the black/black (W = -38, T^+ = 20, T- = -58, P = 0.15) and in the white/white (W = 56, T^+ = 104.5, T- = -48.5, p = 0.19) aquaria on total time measures. In the black/white aquarium more greater time spent was in the black compartment (W = -206, T^+ = 2, T- = -208, $p < 0.01$). Permanence time was also much greater in the black compartment in the black/white aquarium (W = -208, T^+ = 1, T- = -209, $p < 0.01$), but there was no difference in lateral preference in the black/black aquarium (W = -45, T^+ = 23, T- = -68, p = 0.13) or white/white aquarium (W = 44, T^+ = 98.5, T- = -54.5, p = 0.31).

P. axelrodi presented no lateral preference in the black/black aquarium as assessed by total time (W = -13, T^+ = 21, T- = -34, p = 0.56) and permanence time (W = -9, T^+ = 23, T- = 32, p = 0.70); the white / white aquarium generated no

Table 2
Variables of scototaxis in the five teleosts studied (mean±SD) in the proposed text. Refer to text for more information on each variable.

Species	Aquarium	Total time in compartment		Permanence time in compartment		Number of midline crossings	Latency to start exploration
		Black/Left	*White/Right*	*Black/Left*	*White/Right*		
A. altiparanae	Black/black	492,14±262,88	407,86±262,88	133,16±155,99	105,75±150,45	9,63±7,95	374,77±292,98
	White/white	520,61±101,39	387,38±103,67	9,19±3,83	6,42±1,67	62,88±19,95	144,17±139,33
	Black/white	628,46±75,23	333,47±119,73	12,81±5,49	6,15±1,53	56,75±24,18	167,64±131,89
D. rerio	Black/black	675,82±251,40	223,24±252,23	256,49±327,63	56,35±154,39	16,12±19,20	636,92±887,84
	White/white	329,65±321,45	570,35±321,45	117,97±294,02	89,99±214,25	25,47±25,27	156,71±242,59
	Black/white	845,12±92,03	41,29±72,95	208,53±113,45	13,41±17,51	180,21±134,36	544,18±392,52
G. carapo	Black/black	367,59±309,29	528,02±310,27	80,40±148,46	196,60±318,45	9,38±8,75	135,15±292,76
	White/white	323,70±259,86	566,72±264,11	28,38±44,36	78,73±111,75	17,50±11,62	20,34±17,60
	Black/white	626,60±114,68	253,94±100,54	20,45±17,70	6,36±2,70	45,33±22,11	140,34±265,26

O. niloticus, ♂	Black/black	612,11±303,23	265,67±308,63	400,86±414,64	149,28±292,63	7,22±7,53	79,67±101,21
	White/white	310,47±285,40	265,38±270,28	244,67±245,19	231,29±273,14	2,76±2,56	318,85±260,62
	Black/white	894,80±12,26	4,10±11,95	807,00±194,00	4,10±11,95	1,40±0,84	142,10±298,73
O. niloticus, ♀	Black/black	367,17±192,04	498,33±133,88	36,35±24,45	160,81±283,59	17,30±10,21	140,50±202,82
	White/white	320,71±398,79	555,43±384,40	248,93±335,48	498,86±383,62	2,14±1,35	112,71±131,43
	Black/white	697,50±345,71	201,00±345,55	437,42±316,77	192,38±350,12	2,50±2,42	219,40±324,72
P. axelrodi	Black/black	409,50±274,89	490,50±274,89	62,16±96,94	129,15±170,96	11,50±8,50	
	White/white	320,67±263,48	410,67±265,84	8,21±11,55	85,18±175,39	43,33±38,83	
	Black/white	367,23±265,25	364,64±255,16	114,47±235,34	79,31±121,32	179,10±275,87	

lateral preference, either in the total time (W = 1, T+ = 11, T- = -10, p = 1.0) or permanence time measures (W = 5, T+ = 13, T- = 8, p = 0.69). Time spent in the black compartment of the black/white aquarium, though, was much greater than the time spent in the white compartment, as assessed by either total time (W = -118, T+ = 9, T- = -127, p = 0.01) or permanence time (W = -114, T+ = 11, T- = -125, p = 0.002) variables.

In *G. carapo*, there were no differences between total time or permanence time in left or right compartments in the black/black (total time: W = 8, T+ = 22, T- = 14, p = 0.64; permanence time: W = 12, T+ = 24, T- = 12, p = 0.46) and white/white aquaria (total time: W = 6, T+ = 21, T- = -15, p = 0.74; permanence time: W = 6, T+ = 21, T- = -15, p = 0.74). Total time was greater in the black compartment of the black/white aquarium (W = -30, T+ = 3, T- = -33, p = 0.04), but permanence time was not statistically significantly different in between compartments (W = -26, T+ = 5, T- = 31, p = 0.08).

A. altiparanae presented a similar pattern of exploration as the other species; Wilcoxon's signed rank tests for total time in either black/white or left/right compartment in each aquaria resulted as follows: black/black, non-significant (W = -8, T+ = 14, T- = 22, p = 0.64); white/white, non-significant (W = -24, T+ = 6, T- = -30, p = 0.11); black/white, significant (W = -36, T+ = 0, T- = -36, p = 0.01). When permanence time in either black/white or left/right compartments was tested, results were as follows: black/black, non-significant (W = -16, T+ = 6, T- = -22, p = 0.22); white/white, non-significant (W = -24, T+ = 6, T- = -30, p = 0.11); black/white, significant (W = -36, T+ = 0, T- = -36, p = 0.01).

In male Nile tilapias, there was no statistically significant lateral preference in the black/black (total time: W = -29, T+ = 8, T- = -37, p = 0.10; permanence time: W = -29, T+ = 8, T- = -37, p = 0.10) or white/white aquaria (total time: W = -6, T+ = 15, T- = -21, p = 0.74; permanence time: W = -2, T+ = 17, T- = 19, p = 0.945). Animals spent significantly greater time in the black compartment in the black/white aquarium (total time: W = -55, T+ = 0, T- = -55, p = 0.002; permanence time: W = -55, T+ = 0, T- = -55, p = 0.002).

In the preference measures, female *O. niloticus*' behavior was similar to that of males: the black/black aquarium produced no significant lateral preference (total time: W = 9, T+ = 32, T- = -23, p = 0.70; permanence time: W = 15, T+ = 35, T- = -20, p = 0.50). Similarly, neither did the white/white aquarium (total time: W = 6, T+ = 17, T- = 11, p = 0.69; permanence time: W = 8, T+ = 18, T- = -10, p = 0.58). The black/white aquarium produced a consistent and statistically significant preference for dark environments (total time: W = -47, T+ = 4, T- = -51, p = 0.01; permanence time: W = -43, T+ = 6, T- = 49, p = 0.03).

No statistically significant lateral preference in the black/black (total time: W = -1, T+ = 7, T- = -8, p = 1.0; permanence time: W = -1.0, T+ = 7.0, T- = -8.0, p = 1.0) or white/white aquaria (total time: W = -9, T+ = 6, T- = -15, p = 0.44; permanence time: W = -9, T+ = 6, T- = -15, p = 0.44) was observed in male *P. reticulata*. Animals spent significantly greater time in the black compartment in the black/white aquarium (total time: W = -104, T+ = 8, T- = -112, p = 0.002; permanence time: W = -102, T+ = 9, T- = -111, p = 0.002).

Female *P. reticulata* did not present lateral preference in the black/black (total time, W = -9, T_+ = 6, T_- = -15, p = 0.44; permanence time, W = -11, T_+ = 5, T_- = -16, p = 0.31) or white/white aquaria (total time, W = -11, T_+ = 5, T_- = -16, p = 0.31; permanence time, W = -9, T_+ = 6, T_- = -15, p = 0.44); female guppies spent more time in the black compartment of the black/white aquarium (total time: W = -72, T_+ = 24, T_- = -96,000, p = 0.04; permanence time: W = -68, T_+ = 26, T_- = -94, p = 0.06).

Comparative analysis

Since the PHYSIG analysis did not present a statistically significant signal, yielding p-values of 0.76 and 0.37 for traits B:W and AltW respectively (Table 3), the authors opted for a conventional ANOVA approach to compare species. The same variables analysed within-species were analysed between-species; however, since the data presented above demonstrate preference for dark environments in the species studied, only data from the black/white aquarium were used. To facilitate comparison, a proxy variable was made, comprising the ratio of time spent in the black compartment and time spent in the white compartment of the black/white aquarium (B:W). A statistically significant difference between species was found in the B:W variable ($H_{[df=7]}$=17.97 P=0.01). As can be inferred from Table 2, the Cardinal-tetra presented much higher B:W ratios than the other species, while male and female tilapias presented smaller B:W ratios than the other species.

Table 3
Parameters of the PHYSIG phylogenetic signal estimation. K is the ratio between expected MSE_0/MSE and observed MSE_0/MSE with all the parameters set. The tree branches were re-scaled using a transformation parameter d that was estimated by the Ornstein-Uhlenbeck process simulation. Refer to text for more information.

Trait	Expected MSE_0/MSE	Observed MSE_0/MSE	K	Mean MSE permuted data	SD MSE permuted data	Skew MSE permuted data	*p*
B:W	1.13	0.46	0.41	1.53	0.58	-0.09	0.76
AltW	1.13	0.70	0.62	0.54	0.14	0.00	0.37

Discussion

The present data allow us to analyze the parameters of the dark/light preference model in different species of fish. The preference for a dark environment was found in all of the species that were studied, even though they come from different taxa. The pattern of locomotion suggests the white environment is more aversive for those species, with the exception of male and female *O. niloticus* and female *P. reticulata*. The use of redundant variables for both preference and locomotion was intentional; further studies are needed to discriminate whether those variables have differential sensitivity to different treatments, such as parametrical manipulations in the aquaria, rearing conditions, or pharmacological screening. There was also a pattern of sexual dimorphism in

dark/light preference in the Nile tilapia and in the guppy. The magnitude of differences in preference and locomotion variables varies among the species studied, which is consistent with previous experiments with dark/light preference in fishes. This convergence points to species-specific emotional behavior in teleosts (Maximino & Gouveia Jr., *submitted;* Shaklee, 1963).

The observation that animals spent more time in the black environment of the black/white aquarium is representative of preference for dark environments. The greater degree of exploration in the white/white aquarium, as assessed by higher number of midline crossings in this aquarium for the majority of species studied, could be an index of the aversiveness of light environments; this avoidance of bright environments was observed in negative phototaxis experiments (eg, Fernö, Huse, Juell, & Bjordal, 1995; Hafeez & Quay, 1970). It is possible that, if the species did not present greater number of midline crossings, a different pattern of swimming in the white environment was present – for example, a pattern of freezing in the corners of the aquarium (observed in the guppy in Budaev's (1997a) battery of experiments). This is supported by the comparative data on B:W ratios (see below), since those species that presented smaller number of midline crossings in the white/white aquarium also tended to present smaller B:W ratios, an indication that they spent more time in the white environment of the black/white aquarium as well. However, since a detailed observation of the swimming ethogram was not possible, this derivative hypothesis cannot be answered by the present data.

The longer period of latency some animals exhibited to start exploring the white/white aquarium could also be indexical of the aversiveness of this environment, in a manner analogous to the aversiveness of open arms in the rodent elevated plus-maze model (eg., Pellow et al., 1985), a extensively used anxiety model. However, since this measure was statistically different between aquaria in only two species, it should not be suitable for analyses of locomotion and preference in this test. Since the first latency is probably a reaction to confinement stress (cf. Sadler, Pankhurst, Pankhurst, & King, 2000), it is more probable that this heightened latency to start exploring in the white/white aquarium, observed in *D. rerio* and male *O. niloticus*, is an additive effect of the modulation of this stressful/aversive environment and the confinement stress in the animals' exploratory behavior.

The fact that female *O. niloticus* presented consistent preference for dark environments, but their locomotion was heightened by the black/black aquarium, suggests that there is a sexual dimorphism in the behavior of Nile tilapia in the dark/light preference model. This greater locomotion could also be an artifact of the statistical comparisons, since it is possible that female tilapias are more prone to “freezing” in white environments. This effect could also be related to hormonal status, since this is a variable that influences rodent behavior in the dark/light apparatus (Timothy, Costall, & Smythe, 1999), and since teleosts’ brains are very prone to estrogen modulation (e.g. Albert, Crampton, Thorsen, & Lovejoy, 2004; Kim, Stumpf, Sar, & Christine, 1978). There is also the possibility of species differences, unmasked by sex differences, in the pattern of swimming (see below). Female guppies, on the other hand, did not present heightened locomotion in any

of the aquaria. The preference parameters for female *O. niloticus* and *P. reticulata* tend to be more pronounced than those of males from the same species, which could analogous to sexual dimorphism in shyness-boldness.

From a comparative point of view, the present data is very complex. Since no significant phylogenetic signal was found, it is not possible to determine if the species differences observed were due to phylogenetic or other ecological factors. Overall, however, the differences observed in the variable analyzed are not better explained by phylogenetic inertia, i.e., the tendency of closely related species to present similar phenotypes. This can be seen, for example, in the great difference observed in B:W ratios between the characids *P. axelrodi* and *A. altiparanae*. The perciform Nile tilapia, which was the outgroup for the clades chosen, presented a B:W ratio that was very similar to that of the ostariophysi *D. rerio, A. altiparanae* and *G. carapo*. Those differences are also not better explained by differences between wild-caught vs. laboratory-reared animals: recently wild-caught species such as *A. altiparanae* and *G. carapo* presented similar patterns as the other species, with the exception of *P. axelrodi*. Nonetheless, since all species studied presented a similar pattern of scototactic behavior, the present model should be suitable for study of fear- and anxiety-like behavior in teleosts.

It is interesting to notice that, even though *G. carapo* is an weakly electric fish, relying on electric organ discharges (EODs) to orient itself and having vestigial eyes, banded-knife fishes presented a very similar pattern of behavior in the proposed test as other species. *G. carapo* is also bigger than the other species studied, including the perciforms used (*P. reticulata* and *O. niloticus*); since perciforms are highly visually-guided (Kotrschal, van Staaden, & Huber, 1998), it is probable that banded-knife fishes present at least vestigial vision, being able to discriminate contrast.

This pattern of scototactic (ie, darkness-seeking) behavior in different taxa of teleosts could be understood as an adaptation of those species in terms of a crypsis-based defense against predation (e.g. Fuiman & Magurran, 1994; Shaklee, 1963), allowing for the inclusion of this model in the analysis of anxiety/fear systems made by McNaughton & Corr (2004). Prey often respond to a predator's presence (or its possible presence) by increasing the use of refuges (Abrams, 1986, 1984; Blanchard & Blanchard, 1988; Blanchard, et al., 1993; Lima & Dill, 1990); the preference for dark environments observed in the present work is interpreted in this sense. In the bluegill sunfish *Lepomis macrochirus*, the preference for dark environments is conditioned by the light levels in the bright compartment, as well as the presence of a predator in any compartment (McCartt et al., 1997), suggesting that this strategy is based on a trade-off between neophobia/predator avoidance, in one hand, and other environmental variables, including the possibility of crypsis-based defense behavior. The fact that, in the zebrafish, this behavioral pattern is altered by acute treatment with classic antianxiety drugs (Su Guo & Billy Lau, 2006, *personal communication)*, as well as ethanol (Gerlai et al., 2000), presents further support for the proposition that this apparatus generates unconditioned anxiety-like responses in teleosts. Since there are many functional similarities in the central nervous system of teleosts and other vertebrates, at least in the systems that regulate emotional responses (Striedter, 2005), it is probable that the

behavioral pattern found in these species is mediated by monoaminergic and amino acid neurotransmitters (Su Guo & Billy Lau, 2006, *personal communication*).

Taken together, these data demonstrate 1) The presence of a preference for dark environments in the species studied; 2) The aversiveness of the white environment for all species, except *O. niloticus*; 3) Species differences in the preference for darkness; and 4) The suitability of this model for cross-species comparison on "boldness-shyness" and emotional reactivity traits. In conjunction with the observations that antianxiety substances alter the behavior of zebrafish in the black/white box, the authors conclude that this model could be suitable for studying emotion-like behaviors in teleost fishes.

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