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# Same or different? Abstract relational concept use in juvenile bamboo sharks and Malawi cichlids

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## Abstract

Sorting objects and events into categories and concepts is an important cognitive prerequisite that spares an individual the learning of every object or situation encountered in its daily life. Accordingly, specific items are classified in general groups that allow fast responses to novel situations. The present study assessed whether bamboo sharks *Chiloscyllium griseum* and Malawi cichlids *Pseudotropheus zebra* can distinguish sets of stimuli (each stimulus consisting of two abstract, geometric objects) that meet two conceptual preconditions, i.e., (1) "sameness" versus "difference" and (2) a certain spatial arrangement of both objects. In two alternative forced choice experiments, individuals were first trained to choose two different, vertically arranged objects from two different but horizontally arranged ones. Pair discriminations were followed by extensive transfer test experiments. Transfer tests using stimuli consisting of (a) black and gray circles and (b) squares with novel geometric patterns provided conflicting information with respect to the learnt rule "choose two different, vertically arranged objects", thereby investigating (1) the individuals' ability to transfer previously gained knowledge to novel stimuli and (2) the abstract relational concept(s) or rule(s) applied to categorize these novel objects. Present results suggest that the level of processing and usage of both abstract concepts differed considerably between bamboo sharks and Malawi cichlids. Bamboo sharks seemed to combine both concepts—although not with equal but hierarchical prominence—pointing to advanced cognitive capabilities. Conversely, Malawi cichlids had difficulties in discriminating between symbols and failed to apply the acquired training knowledge on new sets of geometric and, in particular, gray-level transfer stimuli.

Key words: visual concept learning, spatial arrangement, relational abstract concept, Malawi cichlid, bamboo shark

Concepts and categories benefit an individual's cognitive economy by obviating the learning of every particular object or situation encountered in its daily life (e.g., Lamberts and Shanks 1997; Mareschal et al. 2010). Instead, any new object or action is immediately placed in a group of similar, already classified items, which allows for prompt identification and therefore quick response to novel situations. Concept learning (also known as category learning or concept attainment) requires the learner to compare and contrast groups or categories that comprise possibly concept-relevant features with groups or categories that do not comprise these features (Bruner et al. 1967). While items within a group or category can vary to some extent, they all must share at least some item-specific (e.g., physical or perceptually similar) features and the development of prototypes, schemas, attributions, or exemplars predictive of the appropriate category (Zentall et al. 2002).

Abstract concepts, in contrast, are not based on item-specific features but on relationships and are therefore not associated with specific stimuli (Newport et al. 2015). To give an example, discrete relations such as "sameness" versus "difference" or "valuable" versus "worthless" can serve as a basis for an abstract grouping of items, situations or ideas, because the relational concept has to be handled independently of the physical or perceptual nature of the objects (e.g., Avarguès-Weber et al. 2012; Newport et al. 2015). In this case, features that can help an individual to designate objects or events as being the "same" or "different" are highly variable and depend on the general context (Newport et al. 2015). Accordingly, the abstract concept of, for instance, "sameness" and "difference" can be applied to a nearly infinite number of ideas or situations. The formation and manipulation of abstract concepts requires a higher level of cognitive reasoning (Katz et al. 2007) and it appears to require time to develop and mature during (human) infancy (Murphy 2004; Mareschal et al. 2010). Therefore, it has long been suggested that only primates are capable of learning this task. Comparative studies investigating the ability of non-primate species to deal with abstract concepts are currently limited to a small group of species including birds (e.g., pigeons: Blaisdell and Cook 2005; Katz and Wright 2006; parrots: Pepperberg 1987), mammals (dolphins: Mercado et al. 2000; sea lions: Kastak and Schusterman; 1994; coati: Chausseil 1991; harbor seals: Scholtyssek et al. 2013; echidna: Russel and Burke 2016), teleost fish (e.g., *Toxotes chatareus*: Newport et al. 2014, 2015), and bees (Giurfa et al. 2001; Avarguès-Weber et al. 2012). While harbor seals, echidna, parrots, and pigeons successfully applied learned rules to novel stimuli, other species, including fish, failed to learn the concept (Newport et al. 2014, 2015).

The general capability to discriminate between a variety of different objects has been thoroughly investigated in bamboo sharks (*Chiloscyllium griseum*, e.g., Fuss et al. 2014a, 2014b, 2017; Fuss and Schluessel 2015, 2017) as well as in Malawi cichlids (*Pseudotropheus zebra*, e.g., Schluessel et al. 2014a, 2015). Juvenile bamboo sharks (Schluessel and Duengen 2015) and Malawi cichlids (Schluessel et al. 2012) distinguished visually between two categories ("fish" and "snail") independently of image features or image type (i.e., black and white drawings, photographs, comics, or negative images) in two alternative forced choice experiments. In this study, bamboo sharks successfully applied the learned rules to novel stimuli. Furthermore, they showed remarkable discrimination and categorization abilities regarding abstract symmetrical and asymmetrical stimulus pairs and showed a spontaneous preference for symmetrical objects (Schluessel et al. 2014b). Contrarily, Malawi cichlids tested in the same study showed no spontaneous preference for symmetry or asymmetry, required many sessions to discriminate between symbols and performed surprisingly poorly in the categorization experiments (Schluessel et al. 2014b) given their outstanding performance in the categorization tasks involving "fish" and "snail" images. Recently, archerfish were successfully trained in a four alternative forced choice experiment (i.e., one stimulus set had two identical, the other two different symbols, one of which the subjects were trained to choose) in which all individuals learned a discrimination test to a high degree of accuracy. However, when the same procedure but variable same and different stimulus pairs were used, only one individual successfully solved the task after 600 training trials and "positive punishment" (i.e., a bitter food reward to increase the fish's motivation to make a correct choice) in 150 additional trials (Newport et al. 2015). However, the ability to process two abstract concepts such as "same" versus "different" and "vertical" versus "horizontal" simultaneously has not been investigated in any fish species yet—neither in a teleost nor in an elasmobranch.

The processing of spatial relationships in natural and artificial environments has been widely examined in various vertebrates and invertebrates, up to complex orientation and navigation studies in the context of cognitive mapping (e.g., Thinus-Blanc 1996; Shapiro 2016). Navigation in three dimensions is crucial in the life of many animals—particularly those that fly or swim—ranging from finding foraging or mating sites to escaping predators. However, most studies focused on how animals orientate in the horizontal plain (Holbrook and de Perera 2009). Only a few studies investigated the ability to develop categorical spatial relationships in a purely perceptual (visual) discrimination task, such as for instance "above" versus "below" or "inside" versus "outside" or "right" versus "left" relations of different stimuli (e.g., lines and dots). For instance, Rufous hummingbirds (Henderson et al. 2006), pigeons (Kirkpatrick-Steger and Wasserman 1996), and primates (baboons: Dépy et al. 1999; chimpanzees: Hopkins and Morris 1989; capuchin monkeys: Spinozzi et al. 2004) showed the ability to form categorical representations of "above" versus "below" or "right" versus "left".

Despite increasing evidence that animals as diverse as primates, birds, teleost fish, and insects are capable of treating different environmental stimuli as "similar" or "different" and of sorting objects and events into categories and concepts, the level of abstraction at which these diverse species develop categories nevertheless remains controversial. Processing two or more concepts at a time presumes an even higher level of cognitive complexity. For instance, Avarguès-Weber et al. (2012) discovered that free-flying honey bees extracted at least two relational concepts from experience with complex stimuli and that these concepts could be combined, independently of the physical nature of the stimuli. All stimuli were (a) arranged in a specific spatial relationship and (b) composed of different visual elements. The authors concluded that "a dual concept based on two distinct relational rules rather than perceptual similarity guided the honey bees' choices in these experiments" (Avarguès-Weber et al. 2012).

The present study investigated for the first time whether Malawi cichlids (*Pseudotropheus zebra*) and gray bamboo sharks (*Chiloscyllium griseum*) can distinguish complex stimuli that meet two prerequisites simultaneously. Individuals were trained to distinguish different stimulus pairs (each stimulus consisting of two objects in a consistent spatial relationship but of different appearance) in four successive training periods. Following successful completion of the training tasks, several transfer tests providing conflicting information examined (1) their ability to transfer previously gained knowledge to novel stimuli and (2) the concept(s) applied to categorize these novel stimuli.

## Material and Methods
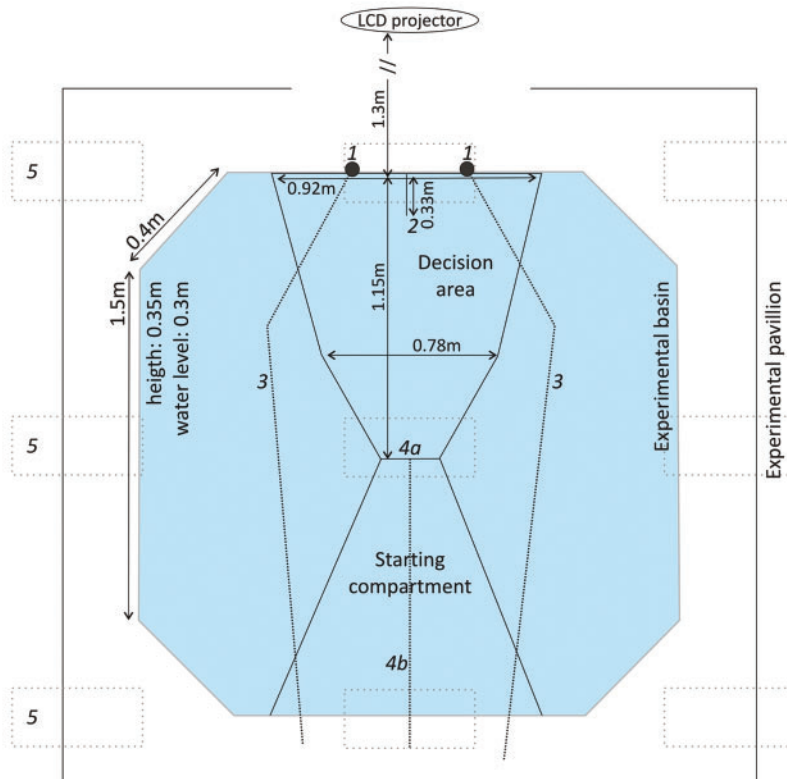
### Group 1—bamboo sharks

#### Animals and housing facilities

Six experimentally experienced female juvenile bamboo sharks (*Chiloscyllium griseum*, TL: 25–40 cm) were kept in one large aquarium ($3.20 \times 1.75 \times 0.60$ m) filled with aerated, filtered salt water [conductance: about 50 mS (ca. 1.0217 kg/dm³) at $26 \pm 2°C$], providing constant environmental conditions (conductivity, temperature, and pH). Additionally, the aquarium was equipped with reef sand and plenty of hiding places.

#### Setup

Shark experiments were performed in a setup situated in an octagonal experimental basin ($2.5 \times 2.5 \times 0.35$ m) (Figure 1A), made out of
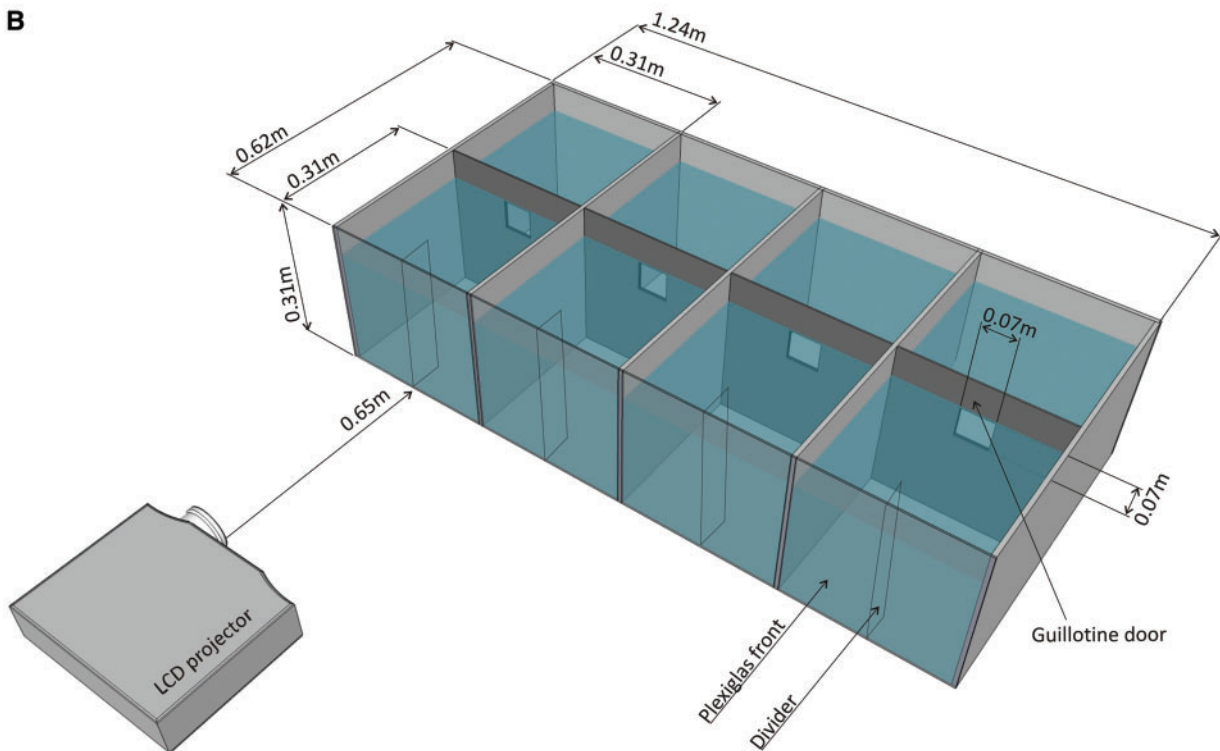
**A**



**B**



**Figure 1.** (**A**) The bamboo sharks' experimental setup within the experimental basin inside a white pavilion. The keyhole-shaped setup consists of a starting compartment, a decision area and a frosted screen for projection with a divider to allow the projection of two 2D objects at a time and to provoke a clear, unambiguous choice (left or right). For projection, an LCD beamer is used. 1 = feeders, 2 = frosted screen for projection, 3 = cable pulls to release food from the feeders, 4a = guillotine door, 4b = cable pull to open guillotine door, 5 = ceiling-mounted fluorescent tubes (above the pavilion roof) (modified from Fuss et al. 2014a). (**B**) The setup used to train cichlids. One of two blocks of four identical tanks divided by partition walls. Each tank consisted of a rear part serving as the main living area and a front part serving as an experimental decision area during training. The LCD projector used to display the stimuli were positioned in front of the frosted acrylic glass front. The entire front wall of a tank was used for the projections (modified from Gierszewski et al. 2013).

transparent acrylic glass and a white floor. The basin was filled with water to a depth of approximately 0.3 m. The basin was surrounded by a white pavilion ($3.0 \times 3.0 \times 2.5$m) to exclude unintentional cueing or disturbing external influences. Ceiling mounted fluorescent tubes provided an even illumination during all experiments (above pavilion roof; Osram L18 W, Lumilux Cool White, Germany). The gray PVC setup (installed within the basin) comprised a triangular starting compartment ($1 \times 1.80 \times 0.35$ m), a decision area ($1.15 \times 0.78 \times 0.35$ m) and a frosted screen for projections ($0.92 \times 0.35$ m) (Figure 1A). Prior to each training trial bamboo sharks were individually transferred to the starting compartment, which was confined by a light gray guillotine door ($0.43 \times 0.23$ m). This door was controlled manually by using a cable/pulley mechanism. A transparent divider ($0.33 \times 0.35$ m) was attached to the frosted screen separating a left from a right division, thereby enforcing an unambiguous decision making in response to the stimuli displayed on the screen (Figure 1A). All stimuli were presented using a LCD projector placed at a distance of 1.3 m from the frosted screen. As bamboo sharks usually swam close to the bottom, stimuli were projected at a height of 3 to 10.5 cm (lowermost part of stimuli, depending on vertical or horizontal arrangement, Figure 2A–E) above the ground. Horizontal feeders were installed just above the stimuli allowing food to be dropped into the setup manually using a cable/pulley mechanism. Both feeders were baited during all trials to exclude unintentional cueing. To prevent bias in the shark's choice in subsequent trials due to any olfactory cues after a reward was given, the water in the maze was stirred after each trial. Upon commencement of the experiments, small pieces of squid, fish, or shrimps were only obtainable during the experimental training. There was a 10 h light: 14 h dark cycle; all experiments were conducted during the light cycle. Individuals were identified based on unique phenotypical characteristics.

## Group 2—Cichlids

### Animals and housing facilities

Eight experimentally experienced Malawi cichlids (*Pseudotropheus zebra*, TL: 6–10 cm) were individually kept in tanks ($0.31 \times 0.31 \times 0.62$ m) made of gray plastic and a frosted acrylic glass front (Figure 1B). Each tank was divided into two compartments allowing entry via a small guillotine door. Apart from the experimental training sessions, this door remained open to allow for sufficient water exchange and to enable individuals to access both compartments. The rear part of each tank served as the main living area and was furnished with fine gravel, a hiding place, an internal water filter, and a small aquarium heater. It also served as a starting compartment for experimental trials. The front part of each tank serves as the experimental decision area. It featured a frosted acrylic glass front for stimulus projections, which was divided into two parts using a small transparent divider, and two feeder brackets (Figure 1B). The aquarium water ($\sim$44 l) was regularly enriched using multivitamins (Atvitol JBL, Germany) and kept at $26 \pm 2°$C, providing constant environmental conditions. Upon commencement of the experiments, food ("granugreen", sera, Germany) was only obtainable during the experimental training. There was a 10 h light: 14 h dark cycle; all experiments were conducted during the light cycle.

### Setup

Prior to the experimental training sessions, hoses containing the reward on one end and a syringe mounted to the other end (altogether serving as feeders) were introduced to the feeder brackets (consisting of a flexible tube on the inside wall) on each side of the divider (Figure 1B). These feeders were installed just above stimuli to reward Malawi cichlids for a correct decision by releasing food manually. Both feeders were baited during all trials to exclude unintentional cueing. Likewise, the internal filter and the aquarium heater were turned off during all trials. The guillotine door was closed and only opened at the beginning of each trial. As Malawi cichlids were usually swimming within the water column, stimuli were projected at a height of 9.5–11 cm above the ground (lowermost part of stimulus, depending on vertical or horizontal arrangement, Figure 2). All stimuli were presented using a LCD projector that was placed at a distance of 0.65 m from the frosted acrylic glass front (Figure 1B).

## General design and procedure

The behavioral experiments for both species (group 1: bamboo sharks, group 2: Malawi cichlids) consisted of two phases: (1) training and (2) transfers tests (Figure 2).

### Phase 1—training

As all individuals had already participated in previous experiments on different visual discrimination tasks (i.e., bamboo sharks: Fuss et al. 2017, Malawi cichlids: Schluessel et al. 2018), they were used to the setup, training and feeding procedure. Before training, bamboo sharks were gently caught manually and transferred from their home tank into the experimental setup. Malawi cichlids were gently guided into the rear part of their housing tanks and the guillotine door was closed. No evidence of stress due to handling was observed.

To start a training trial, the guillotine door was raised. After leaving the SC, the individual was allowed to move freely within the decision area and to choose the viewing distance that best meets its visual abilities. During regular training trials, the two stimuli to be discriminated were displayed simultaneously (one each in the left and right division) and switched randomly between the left and the right side of the screen to avoid any kind of direction conditioning (Figures 1A–B and 2A). To vary the succession of stimuli shown on a particular side across sessions, five alternating rotational schemes were used. To indicate a choice within the maximum trial duration of two min, bamboo sharks had to touch the "target" (i.e., represented by a cross in the centre of each stimulus, Figure 2) with their snout. Similarly, Malawi cichlids had to pass a virtual decision line with their pectoral fins about five cm in front of the presented stimuli (Figure 1B). A correct choice was rewarded with food. During the inter-trial-interval, the individual was allowed to swim freely throughout the entire setup for 30 s, before it was gently guided back into the starting compartment. An incorrect choice resulted in a black screen and an immediate return to the starting compartment. If an individual did not choose within the allocated 2 min, this trial was terminated.

Training sessions were carried out 5 days per week for bamboo sharks (one training session per day) and 6 days per week for Malawi cichlids (two training sessions per day); each session consisting of 12 trials per individual. Training was completed as soon as the learning criterion of $\geq$75% correct choices in five out of six consecutive training sessions [$\chi^2$ (1) $\leq 0.05$ for statistical significance] was reached. If an individual did not reach the criterion within 40 training sessions in Training 1, training was terminated and the individual continued with Training 2 (Figure 2A). Since individuals that repeatedly failed to solve a task successively showed severe
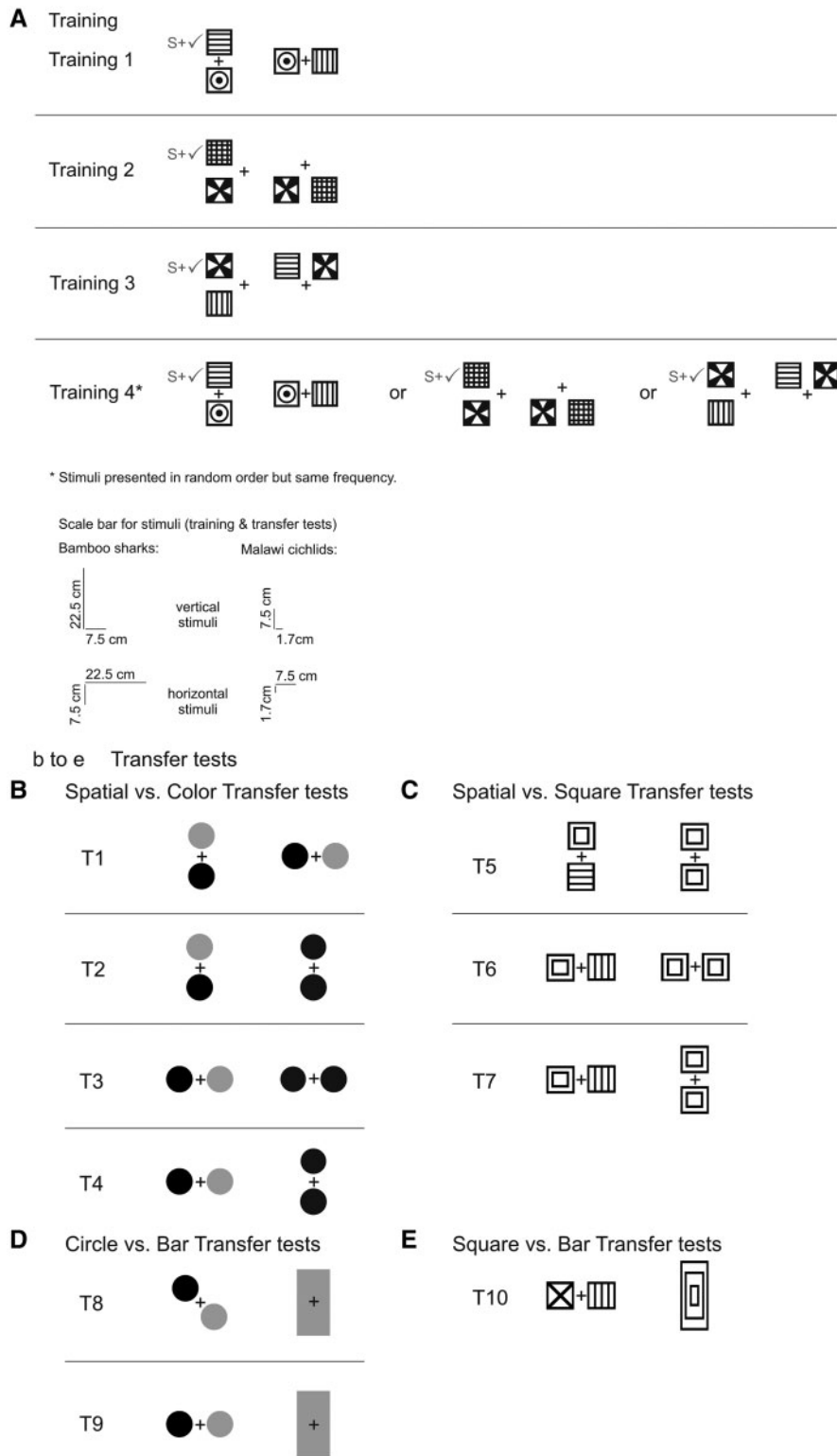
**Figure 2.** Stimuli presented during training and transfer tests T1–T10. **(A)** During regular training trials, the two stimuli to be discriminated were displayed simultaneously (one in each division) and switched randomly between the left and the right side of the screen. While only one stimulus pair was used at a time until an individual reached the learning criterion during Training 1–3, all three previously learned stimulus pairs were randomly presented in the fourth training task (same frequence, but with the sequence selected at random). During Training 1 to 4, the two different, vertically arranged objects of each stimulus set was defined as the positive, rewarded stimulus (indicated by 'S+✓'). **(B–E)** A transfer stimulus consisted of two objects either of black and gray circles, squares with geometric patterns, or a single vertical bar. Again, both stimuli to be discriminated were displayed simultaneously and switched randomly between the left and the right side of the screen. Transfer test stimuli covered the same dimensions as training stimuli. Stimulus sizes for bamboo sharks' and Malawi cichlids' stimuli are indicated by scale bars.

motivational deficits to the point of refusal to participate, the maximum number of training sessions was reduced to 30 in Training 2, 3 or 4. Accordingly, if an individual failed to reach the criterion within 30 training sessions for a second time, it was excluded from further training.

### Training stimuli

Initially, only one stimulus pair was used at a time until an individual reached the learning criterion. The same procedure was followed in the next two training tasks, but each comprising new stimulus pairs. Thus, Training 1 to 3 (Figure 2A) allowed individuals to solve the task using direct association between the positive stimulus and a food reward. In the fourth training task, all three previously learned stimulus pairs were then randomly presented to encourage individuals to generalize their previously gained knowledge.

Each training stimulus consisted of two objects (i.e., squares with different geometric patterns; Figure 2A). In addition, the stimuli presented to bamboo sharks confined a "target" (i.e., a cross), serving as a focal point to draw their attention to both objects forming a stimulus (Figure 2). Since the "target" deterred the Malawi cichlids from approaching the decision line in the initial training sessions, it was removed from their stimuli. The vertical stimulus was always defined as the positive (i.e., rewarded) one.

To facilitate reading in the following sections, stimuli comprising two different objects will be referred to as "2 D stimuli", while stimuli comprising 2 similar objects will be referred to as "2S stimuli".

### Phase 2—transfers

As soon as an individual successfully passed the training phase, the transfer phase commenced. Ten different transfer tests (i.e., T1 to T10, Figure 2B–E) were conducted during which the individuals had to perform under altered conditions to estimate which categories, rules, or concepts the individuals might have associated with the training stimuli (hereafter referred to as the "training paradigm").

With respect to the training paradigm ("choose the vertical stimulus and neglect the horizontal alternative"), individuals could either rely on (a) the spatial arrangement of the objects forming a stimulus (i.e., vertical/horizontal), (b) the objects' appearance (two similar or two different objects or one bar), or (c) a combination of both features in the transfer tests. As conflicting information was provided during selected transfer tests, forcing individuals to decide in favor of one strategy over another, option (c) was expected to confuse the individuals, causing them to choose according to chance.

Up to four transfer trials were interspersed randomly with 12 regular training trials (resulting in 16 trials per session). Each transfer test T1–T10 was presented 20 times to every individual and were interspersed randomly (i.e., same frequence, but with the sequence selected at random).

Transfer trials remained unrewarded to prevent any kind of learning with respect to the new stimuli (Figure 2B–E). Prior to the first transfer test but following completion of phase 1, individuals were prepared for this occurrence by occasionally withholding reward during regular trials (i.e., two out of twelve randomly chosen regular trials remained unrewarded, irrespective of the individual's choice). This served to keep individuals from recognizing that only transfer trials were unrewarded and therefore not worth participating in. This rewarding scheme was maintained throughout the remainder of the transfer phase.

### Transfer test stimuli

A transfer stimulus consisted of two objects, either of black and gray circles (transfer tests T1 – T4, T8 – T9) or squares with geometric patterns (transfer tests T5 – T7, T10). In transfer tests T8–T10, there was always one stimulus consisting of a single vertical bar. Transfer test stimuli had the same physical dimensions as training stimuli.

### Transfer tests T1–T4 (Spatial versus Color transfer tests)

Transfer tests T1 to T4 tested the individuals' response to stimuli featuring black and gray circles (Figure 2B). T1 trials resembled the training task, but now using black and gray circles. With respect to the training paradigm conflicting information was provided regarding the objects' appearance and the objects' spatial arrangement within the stimuli [i.e., two vertical stimulus pairs (T2), two horizontal stimulus pairs (T3) or two different horizontal versus two similar vertical objects (T4)]. Individuals were expected to choose the stimulus comprising two different ("2D") objects (arranged vertically in T1 and T2, horizontally in T3 and T4 trials), while also choosing on the basis of specific spatial relationships.

### Transfer tests T5–T7 (Spatial versus Geometry transfer tests)

Transfer tests T5–T7 tested the individuals' response to stimuli featuring squares with novel patterns (Figure 2C). These stimuli consisted of either two different or two similar squares with geometric patterns. Individuals were expected to choose the stimulus comprising two different objects (arranged vertically in T5, horizontally in T6 and T7 trials).

### Transfer tests T8–T9 (Circle versus Bar transfer tests) and T10 (Square versus Bar transfer tests)

T8 and T9 stimuli consisted of a gray and a black circle against a single gray bar; T10 stimulus pairs consisted of two different squares with geometric patterns against a single bar. These tests investigated the response to conflicting information on the objects' spatial arrangement and the objects' appearance [i.e., diagonal orientation (T8), horizontal orientation (T9) versus a single gray vertical bar]. Individuals were expected to choose the stimulus comprising two different objects.

## Data analysis

For both experiments, the percentage of correct choices and the average trial time were recorded for each session for each individual. The learning criterion was set to be $\geq 75\%$ correct choices in five out of six consecutive training sessions ($\chi^2 \leq 0.05$, $df = 1$).

Each transfer test T1–T10 was presented 20 times to every individual in the same frequency, but with the sequence selected randomly. One-tailed binomial tests were calculated for each individual to determine which stimuli individuals preferred significantly (i.e., "2D stimuli" over "2S stimuli" or "2D stimuli" over a single bar) within the respective series of 20 transfer trials per test. Additionally, one-tailed binomial tests were calculated on combined results of "spatial *versus* color" transfer tests T1–T4, "spatial *versus* geometry" transfer tests T5–T7, and "circle *versus* bar" and "square *versus* bar" transfer tests T8–T10 for every individual (including every 20 trials per transfer test). Additionally, the individual's choice in the very first trial per transfer test (Tables 2–4) is given. To show that the individual's transfer performance was stable across the series of 20 transfer trials for each transfer test T1–T10, choice consistency was examined using a "mixed logistic regression" model (MLM), whose random effect was individual ID, to estimate the

individual choice probabilities during the transfer trials (for full individual results see Supplementary Table).

For all tests a $P \leq 0.05$ was considered significant, a $P \leq 0.001$ highly significant. Additionally, the Holm–Bonferroni procedure was applied to correct the level of significance for multiple comparisons to $P_{\alpha 1} \leq 0.01$.

## Results

Five out of six bamboo sharks finished the training procedure including all transfer trials (Table 1). As Shark 6 failed to reach the learning criterion in Training 1 and refused to participate in the following training sessions, it was excluded from further training.

Five out of eight Malawi cichlids finished the training procedure including all transfer trials (Table 1). While Malawi cichlids 6 and 7 failed to reach the learning criterion in two training tasks, Malawi cichlid 8 successfully solved Training 1–3 within 10–25 training sessions but, for no apparent reason, it refused to participate after 23 sessions in Training 4. Therefore, Malawi cichlids 6, 7, and 8 were excluded from further experiments. Accordingly, the following only refers to the successful individuals (i.e., $n_{\text{Bamboo sharks}} = 5$, $n_{\text{Malawi cichlids}} = 5$).

## Training

Five sharks reached the learning criterion on average within $14.8 \pm 6.31$ training sessions in Training 1. While the number of sessions considerably decreased during Training 2–3 (Table 1), it increased again in Training 4, during which all learnt stimuli were randomly presented (Figure 2A, Table 1). Although Shark 6 did not reach the pre-established learning criterion within the allocated 40 training sessions in Training 1, it nevertheless chose the positive stimulus significantly more often over the incorrect one (i.e., 74.33% correct choices, $P \leq 0.001$).

Four Malawi cichlids reached the learning criterion on average within $32 \pm 3.94$ training sessions in Training 1. While only two Malawi cichlids reached the learning criterion in Training 2, the number of training sessions of all five successful individuals considerably decreased during Training 3 and 4 (Table 1). Although

**Table 1.** Individual and group training performance of sharks and cichlids. Sessions to reach the learning criterion during training 1–4 for every individual as well as the group (confining the successful individuals $n_{\text{sharks}} = 5$, $n_{\text{cichlids}} = 5$).

| Subject | Sessions to reach learning criterion | | | |
|---|---|---|---|---|
| | Training 1 | Training 2 | Training 3 | Training 4 |
| Shark 1 | 22 | 10 | 8 | 16 |
| Shark 2 | 22 | 6 | 7 | 6 |
| Shark 3 | 9 | 12 | 8 | 16 |
| Sharks 4 | 14 | 9 | 10 | 7 |
| Shark 5 | 7 | 10 | 6 | 10 |
| Shark 6[a] | > 40[a] | – | – | – |
| Group (Sharks 1–5) | $14.8 \pm 6.31$ | $9.4 \pm 1.96$ | $7.6 \pm 1.50$ | $11 \pm 4.29$ |
| Cichlid 1 | 35 | 40 | 6 | 6 |
| Cichlid 2 | 26 | >40 | 9 | 6 |
| Cichlid 3 | 36 | >40 | 19 | 6 |
| Cichlid 4 | 31 | >40 | 18 | 20 |
| Cichlid 5 | >40 | 29 | 20 | 28 |
| Cichlid 6[b] | 24 | >40 | >30 | – |
| Cichlid 7[b] | >40 | >30 | – | – |
| Cichlid 8[b] | 25 | 24 | 10 | (23) |
| Group (Cichlids 1–5) | $32 \pm 3.94$ | $34.5 \pm 5.50$ | $14.4 \pm 5.75$ | $13.2 \pm 9.17$ |

[a] Shark 6 did not reach the learning criterion within the allocated training sessions, refused to participate in Training 2 and, therefore, was excluded from training.
[b] Cichlids did not reach the learning criterion within the allocated training sessions, and, therefore, were excluded from training. Cichlid 8 refused to participate in Training 4 after 23 training sessions and, therefore, was excluded from further training.

**Table 2.** Results of spatial *versus* color transfer tests. The individuals' choice in the very first trial per transfer test type and one-tailed binomial test results considering all 20 trials per individual are given for T1–T4 transfer tests. Additionally, a summary considering all T1–T4 trials per individual (i.e., 80 trials per individual) is given.

| Subject | → | Shark 1 | Shark 2 | Shark 3 | Shark 4 | Shark 5 | Group (choice: "2D stimuli") |
|---|---|---|---|---|---|---|---|
| T1 | Choice in trial 1 | Horizontal | Horizontal | Vertical | Vertical | Vertical | 52% |
| | 1-Tailed binomial test | 0.421 | 0.058 | 0.006**,# | 0.252 | 0.132 | $10.4 \pm 3.61$ |
| T2 | Choice in trial 1 | 2D | 2D | 2D | 2S | 2D | 73% |
| | 1-Tailed binomial test | 0.021* | 0.021*,# | 0.001***,# | 0.412 | 0.021* | $14.6 \pm 1.95$ |
| T3 | Choice in trial 1 | 2D | 2D | 2D | 2D | 2S | 77% |
| | 1-Tailed binomial test | <0.001***,# | 0.021* | 0.001***,# | 0.412 | 0.006**,# | $15.4 \pm 2.42$ |
| T4 | Choice in trial 1 | 2D | 2D | 2D | 2D | 2D | 71% |
| | 1-Tailed binomial test | 0.006***,# | 0.252 | 0.021* | 0.132 | 0.021* | $14.2 \pm 1.47$ |
| Summary T1–T4 | 1-Tailed binomial test | <0.001***,# | <0.001***,# | 0.002**,# | 0.028* | 0.005**,# | |

| | → | Cichlid 1 | Cichlid 2 | Cichlid 3 | Cichlid 4 | Cichlid 5 | Group (choice: "2D stimuli") |
|---|---|---|---|---|---|---|---|
| T1 | Choice in trial 1 | Horizontal | Horizontal | Horizontal | Horizontal | Horizontal | 65% |
| | 1-Tailed binomial test | 0.252 | 0.132 | 0.132 | 0.132 | 0.058 | $13 \pm 0.63$ |
| T2 | Choice in trial 1 | 2S | 2D | 2D | 2S | 2S | 51% |
| | 1-Tailed binomial test | 0.412 | 0.412 | 0.588 | 0.588 | 0.412 | $10.2 \pm 0.75$ |
| T3 | Choice in trial 1 | 2D | 2D | 2D | 2S | 2D | 58% |
| | 1-Tailed binomial test | 0.252 | 0.252 | 0.132 | 0.412 | 0.252 | $11.6 \pm 1.36$ |
| T4 | Choice in trial 1 | 2S | 2S | 2D | 2S | 2D | 46% |
| | 1-Tailed binomial test | 0.132 | 0.412 | 0.252 | 0.058 | 0.058 | $9.2 \pm 2.92$ |
| Summary T1 to T4 | 1-Tailed binomial test | 0.367 | 0.455 | 0.367 | 0.046* | 0.455 | |

**Table 3.** Results of spatial versus geometry transfer tests. The individuals' choice in the very first trial per transfer test type and 1-tailed binomial test results considering all 20 trials per individual are given for T5–T7 transfer tests. Additionally, a summary considering all T5–T7 trials per individual (i.e., 60 trials per individual) is given.

| Subject | $\rightarrow$ | Shark 1 | Shark 2 | Shark 3 | Shark 4 | Shark 5 | Group (choice: '2D stimuli') | |
|---|---|---|---|---|---|---|---|---|
| T5 | Choice in trial 1 | 2D | 2D | 2D | 2S | 2D | 81% | |
| | 1-Tailed binomial test | 0.006**,# | <0.001***,# | 0.006**,# | 0.021* | 0.006**,# | 16.2 ± 0.98 | |
| T6 | Choice in trial 1 | 2S | 2D | 2D | 2D | 2D | 67% | |
| | 1-Tailed binomial test | 0.021* | 0.058 | 0.006**,# | 0.006**,# | 0.058 | 13.4 ± 3.78 | |
| T7 | Choice in trial 1 | 2D | 2D | 2D | 2D | 2D | 62% | |
| | 1-tailed binomial test | 0.058 | 0.006**,# | 0.058 | 0.252 | <0.001***,# | 12.4 ± 4.63 | |
| Summary T5–T7 | 1-Tailed binomial test | <0.001***,# | <0.001***,# | 0.026*,# | 0.014*,# | <0.001***,# | | |
| | $\rightarrow$ | Cichlid 1 | Cichlid 2 | Cichlid 3 | Cichlid 4 | Cichlid 5 | Group (choice: '2D stimuli') | |
| T5 | Choice in trial 1 | 2S | 2S | 2S | 2D | 2D | 73% | |
| | 1-Tailed binomial test | 0.006**, # | 0.006**, # | 0.132 | 0.001**, # | 0.412 | 14.6 ± 2.25 | |
| T6 | Choice in trial 1 | 2S | 2S | 2D | 2D | 2D | 36% | |
| | 1-Tailed binomial test | 0.056 | ≤0.001**, # | 0.132 | 0.058 | 0.412 | 7.2 ± 3.65 | |
| T7 | Choice in trial 1 | 2D | 2S | 2D | 2S | 2S | 37% | |
| | 1-tailed binomial test | 0.412 | 0.006**, # | 0.412 | 0.058 | 0.412 | 7.4 ± 2.06 | |
| Summary T5–T7 | 1-Tailed binomial test | 0.449 | 0.026* | 0.122 | 0.449 | 0.449 | | |

\*$P \leq 0.05$; \*\*$P \leq 0.01$; \*\*\*$P \leq 0.001$; Level of significance according to Holm–Bonferroni correction ($n = 5$): #$P_{\alpha 1} \leq 0.01$. ... Individual preferred to choose "2 similar objects".

**Table 4** Shark Results of Circle *vs*. Bar and Square *vs*. Bar Transfer tests. The individuals' choice in the very first trial per transfer test type and 1-tailed binomial test results considering all 20 trials per individual are given for T8 to T10 transfer tests. Additionally, a summary considering all T8 to T10 trials per individual (i.e., 60 trials per individual) is given.

| Subject | $\rightarrow$ | Shark 1 | Shark 2 | Shark 3 | Shark 4 | Shark 5 | Group (choice: '2D stimuli') | |
|---|---|---|---|---|---|---|---|---|
| T8 | Choice in trial 1 | 2D | 2D | 2D | 2D | 2D | 73% | |
| | 1-Tailed binomial test | 0.132 | 0.058 | 0.001***,# | 0.132 | 0.006**,# | 14.6 ± 1.63 | |
| T9 | Choice in trial 1 | 2D | 2D | 2D | Bar | 2D | 65% | |
| | 1-Tailed binomial test | <0.001***,# | 0.132 | 0.021* | 0.006**,# | 0.021* | 13 ± 4.78 | |
| T10 | Choice in trial 1 | 2D | 2D | Bar | 2D | 2D | 60% | |
| | 1-Tailed binomial test | 0.412 | 0.001***,# | 0.412 | 0.588 | 0.132 | 12 ± 2.83 | |
| Summary T8–T10 | 1-Tailed binomial test | 0.001***,# | <0.001***,# | 0.003**,# | 0.259 | <0.001***,# | | |
| | $\rightarrow$ | Cichlid 1 | Cichlid 2 | Cichlid 3 | Cichlid 4 | Cichlid 5 | Group (choice: '2D stimuli') | |
| T8 | Choice in trial 1 | Bar | Bar | 2D | Bar | Bar | 55% | |
| | 1-Tailed binomial test | 0.588 | 0.132 | 0.412 | 0.132 | 0.058 | 10.2 ± 2.04 | |
| T9 | Choice in trial 1 | 2D | Bar | 2D | 2D | 2D | 51%10.6 ± 2.58 | |
| | 1-Tailed binomial test | 0.588 | 0.132 | 0.412 | 0.132 | 0.058 | | |
| T10 | Choice in trial 1 | 2D | 2D | 2D | 2D | Bar | 85% | |
| | 1-Tailed binomial test | ≤0.001**, # | ≤0.001**, # | 0.001**, # | 0.001**, # | 0.001**, # | 13.4 ± 6.25 | |
| Summary T8–T10 | 1-tailed binomial test | 0.349 | <0.001***,# | <0.001***,# | <0.001***,# | 0.001***,# | | |

\*$P \leq 0.05$, \*\*$P \leq 0.01$, \*\*\*$P \leq 0.001$ significant; level of significance according to Holm–Bonferroni correction ($n = 5$): #$P_{\alpha 1} \leq 0.01$., ... Individual preferred to choose "2 similar objects".

Cichlid 6 did not reach the pre-established learning criterion in Training 2 and 3, it nevertheless chose the positive stimulus significantly more often over the incorrect one in Training 3 (i.e., 62.47% correct choices, $P \leq 0.001$).

During the following transfer test phase, all participating individuals showed very high levels of performance during regular training trials (bamboo sharks: 78.70% ± 0.09 correct decisions, Malawi cichlids: 82% ± 0.11 correct decisions).
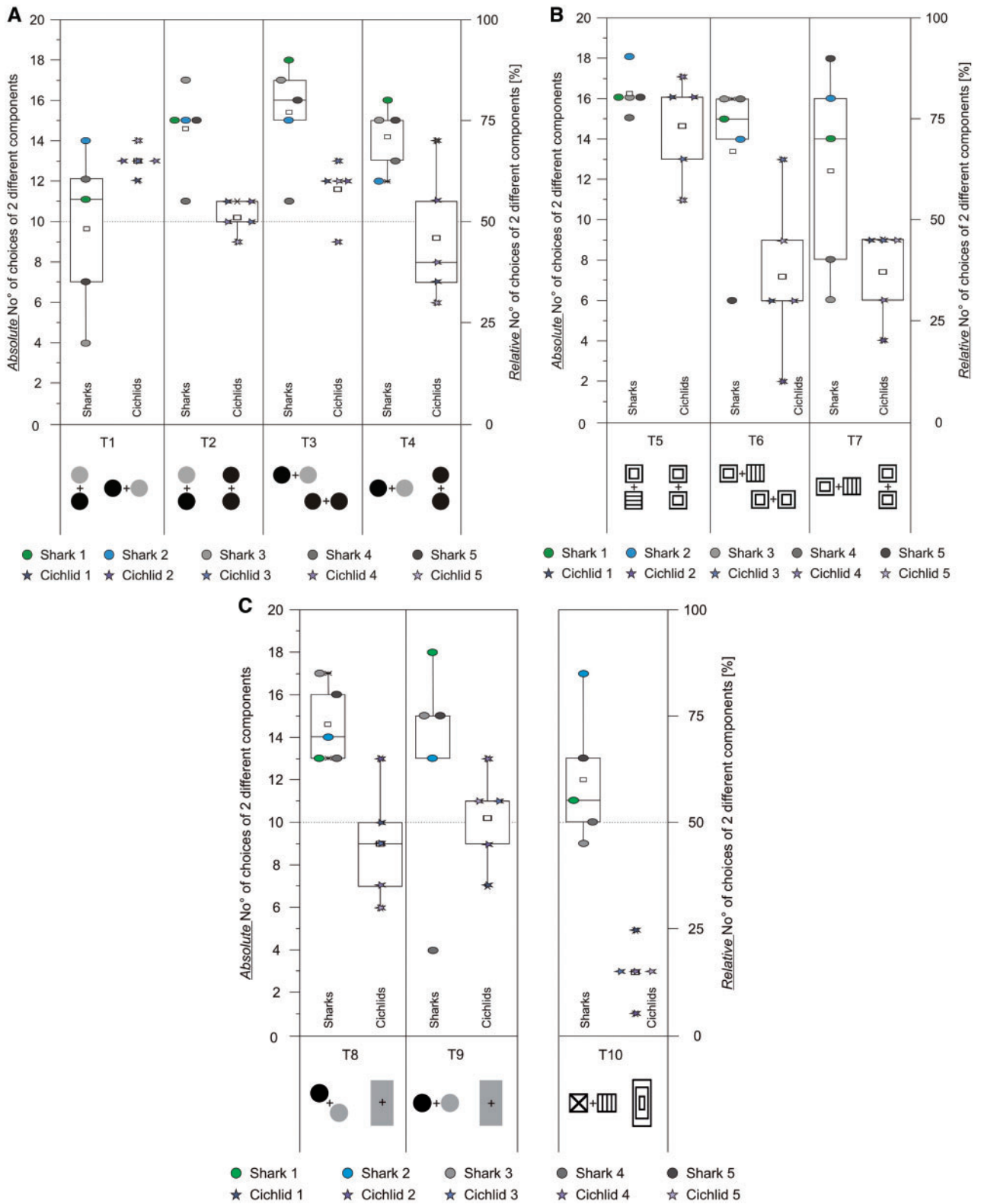
**Figure 3.** Boxplots of individual results, variance and median of bamboo sharks and Malawi cichlids for **(A)** transfer tests T1–T4, **(B)** transfer tests T5–T7 and **(C)** transfer tests T8–T10, indicating the individual's absolute number of choices (left ordinate) and the individual's relative number of choices in [%] (right ordinate). The sharks' results are indicated by circles, the cichlids' results are indicated by asterisks (including color coding per individual). The stimuli tested during T1–T10 are shown on the x-axis.

### Transfer tests T1–T4 (Spatial versus Color transfer tests)

In the T1 transfer test, three bamboo sharks chose the horizontal and two bamboo sharks the vertical stimulus in the very first trial (MLM on choice consistency: range of $\chi^2 = 8.454$ to $3.373$; all $P \geq 0.066$; $df = 1$). One shark significantly preferred the combination resembling the training tasks; additionally, another one showed a distinct tendency (Table 2, Figure 3A) to choose the "2D" stimulus. All Malawi cichlids preferred the horizontal stimulus in the very first T1 trial (MLM on choice consistency: range of $\chi^2 = 0.006$ to $0.281$; all $P \geq 0.596$; $df = 1$), but all individuals decided indifferently in the series of 20 trials (Table 2, Figure 3A).

In T2 and T3 transfer tests (conflicting information between stimulus appearance and spatial arrangement), four bamboo sharks chose the "2D" stimuli in the very first trial in both transfer tests (MLM on choice consistency T2: range of $\chi^2 = 0.002$ to $1.364$; all $P \geq 0.243$; $df = 1$; T3: range of $\chi^2 = 0.391$ to $2.946$; all $P \geq 0.086$; $df = 1$). Four bamboo sharks significantly preferred the "2D" stimuli in the series of 20 trials ($P \leq 0.01$, Table 2, Figure 3A). While two Malawi cichlids chose the "2D" stimuli in the very first T2 trial, four chose "2D" in the first T3 trial (MLM on choice consistency T2: range of $\chi^2 = 0.006$ to $2.940$; all $P \geq 0.086$; $df = 1$; T3: range of $\chi^2 = 0.025$ to $1.647$; all $P \geq 0.199$; $df = 1$), but all individuals chose indifferently between both alternatives in both series of transfer trials (Table 2, Figure 3A).

In T4 transfer trials, all bamboo sharks chose the "2D" stimuli in the very first trial (MLM on choice consistency: range of $\chi^2 = 0.002$ to $0.964$; all $P \geq 0.326$; $df = 1$). Three bamboo sharks appeared to consider the objects' appearance (i.e., "2D" stimuli) to be more important, while two chose indifferently between both alternatives in this series of 20 trials (Table 2, Figure 3A). Two Malawi cichlids chose the "2D" stimuli in the very first T4 trial (MLM on choice consistency: range of $\chi^2 = 0.007$ to $0.442$; all $P \geq 0.506$; $df = 1$), but all individuals chose indifferently between both alternatives in this series of transfer trials (i.e., "2D" stimuli or "2S" stimuli or "bar") (Table 2, Figure 3A).

Summarizing the T1–T4 results, all bamboo sharks significantly preferred the "2D" stimuli, while Malawi cichlids chose indifferently between both alternatives or preferred the "2S" stimuli.

### Transfer tests T5–T7 (Spatial versus Geometry transfer tests)

Four bamboo sharks chose the "2D" stimuli in the very first T5 and T6 trial (MLM on choice consistency T5: range of $\chi^2 = 0.009$ to $1.777$; all $P \geq 0.378$; $df = 1$; T6: range of $\chi^2 = 0.017$–$1.866$; all $P \geq 0.172$; $df = 1$). All bamboo sharks significantly preferred the "2D" stimulus in the series of 20 T5 trials ($P \leq 0.01$, Table 3, Figure 3B). Similarly, while three bamboo sharks significantly preferred the "2D" stimulus in T6 trials, two others showed a tendency to choose the same stimulus (Table 3, Figure 3B). In the very first T5 and T6 trials, two to three Malawi cichlids chose the "2D stimuli" (MLM on choice consistency T5: range of $\chi^2 = 0.009$ to $2.739$; all $P \geq 0.098$; $df = 1$; T6: range of $\chi^2 = 0.002$ to $0.462$; all $P \geq 0.496$; $df = 1$). While three Malawi cichlids chose the "2D" stimulus significantly more often in T5 trials ($P \leq 0.01$, Table 3), three individuals clearly preferred to choose the "2S" stimuli in T6 trials.

All bamboo sharks chose the "2D" stimuli in the very first T7 trial (MLM on choice consistency: range of $\chi^2 = 0.018$ to $0.236$; all $P \geq 0.627$; $df = 1$). While two sharks appeared to consider the objects' appearance (i.e., "2D" stimulus) to be more important, two others showed a tendency to choose the same stimulus in the series of 20 trials (Table 3, Figure 3B). Two Malawi cichlids chose the "2D" stimuli in the very first T7 trials (MLM on choice consistency:

range of $\chi^2 = 0.074$ to $1.052$; all $P \geq 0.305$; $df = 1$). One individual significantly preferred the "2S" stimulus in T7 transfer trials; the others chose indifferently (Table 3, Figure 3B).

Summarizing the T5–T7 results, all bamboo sharks significantly preferred the "2D" stimuli, while Malawi cichlids chose indifferently between both alternatives or preferred the "2S" stimuli.

### Transfer tests T8–T9 (Circle versus Bar transfer tests) and T10 (Square versus Bar transfer tests)

All bamboo sharks chose the "2D" stimuli in the very first T8 trial (MLM on choice consistency: range of $\chi^2 = 0.043$ to $2.739$; all $P \geq 0.098$; $df = 1$). Four bamboo sharks chose the "2D" stimuli in the very first T9 trial (MLM on choice consistency: range of $\chi^2 = 0.002$ to $1.135$; all $P \geq 0.287$; $df = 1$). While two sharks significantly preferred the "2D" stimulus, one bamboo shark only showed a similar tendency to choose this stimulus in T8 trials (Table 4, Figure 3C). Similarly, four bamboo sharks appeared to focus on the object's appearance and significantly preferred the "2D" stimulus in the series of T9 trials (Table 4, Figure 3C). Four Malawi cichlids chose the "bar" stimulus in the very first T8 trial (MLM on choice consistency: range of $\chi^2 = 0.074$ to $0.553$; all $P \geq 0.457$; $df = 1$), but all individuals decided indifferently between both alternatives in the series of 20 trials (Table 4, Figure 3C). In T9 transfer trials, four Malawi cichlids chose the "2D" stimulus in the very first trial (MLM on choice consistency: range of $\chi^2 = 0.002$ to $2.661$; all $P \geq 0.103$; $df = 1$), but all individuals chose indifferently between both alternatives (Table 4, Figure 3C).

Four bamboo sharks chose the "2D" stimuli in the very first T10 trial (MLM on choice consistency: range of $\chi^2 = 0.258$ to $1.382$; all $P \geq 0.239$; $df = 1$). While four bamboo sharks chose indifferently between both alternatives, one individual highly significantly preferred the "2D" stimulus in the series of 20 trials (Table 4, Figure 3C). Four Malawi cichlids chose the "2D" stimulus in the very first T10 trial (MLM on choice consistency: range of $\chi^2 = 0.018$ to $2.338$; all $P \geq 0.126$; $df = 1$) and all Malawi cichlids significantly preferred the "2D" stimulus (all $P \leq 0.001$, Table 4, Figure 3C).

Summarizing the T8–T10 results, four bamboo sharks and three Malawi cichlids significantly preferred the "2D" stimuli. While one cichlid chose indifferently between both alternatives, another one significantly preferred the "2S" stimuli.

## Discussion

The present study investigated whether juvenile bamboo sharks and Malawi cichlids can learn two abstract concepts at the same time using two alternative forced choice experiments. Five out of six bamboo sharks and five out of eight Malawi cichlids finished the training procedure. Contrary to expectations, Malawi cichlids performed poorly during training, needing up to 36 sessions and not all tasks were mastered by each individual. To investigate whether the individuals could transfer their knowledge gained during training—that is both objects must be different while also choosing based on specific (horizontal or vertical) spatial arrangements—several transfer tests were performed. Remarkably, only one bamboo shark but none of the Malawi cichlids succeeded in the T1 trials using novel gray and black circles. In transfer tests, T2–T4 (spatial versus color transfer tests) and T5–T7 (spatial versus geometry transfer tests), individuals were expected to choose the stimulus comprising two different objects ("2D") while also choosing on the basis of specific spatial relationships. With few exceptions, bamboo sharks

spontaneously preferred the "2D" stimuli in their very first trials of these transfer tests. In contrast, T5–T7 transfer tests did not significantly reveal any particular choice strategy in Malawi cichlids, which appeared to choose randomly in these transfer test trials. However, considering that random choice may be associated with exploration preceding any particular decision-making strategy, further research is needed to identify the patterns of choices deployed in Malawi cichlids. Individuals were also tested for their preference of either object similarity or their relative configuration or, alternatively, other, maybe broader cues such as the general alignment of a stimulus. Bamboo sharks preferred two different gray and black circles to a single gray bar if they were arranged horizontally, the deemed wrong orientation (T8/T9), and if they were displayed in the appropriate vertical orientation (T2/T5). Surprisingly, they used the same strategy when presented with the same task involving achromatic squares (T5), but a different strategy when presented with squares versus a bar (T10; except for one shark). Again, Malawi cichlids chose according to chance when being presented with two different gray and black circles (T8/T9), but significantly preferred the two different objects when presented with different squares (T10).

The Malawi cichlids' training performance was unexpectedly poor, because they had already shown pronounced cognitive performances in previous studies (e.g., Schluessel et al. 2012, 2014a,b). Several earlier studies also suggested that Malawi cichlids rely heavily on their visual system for a variety of activities including mate choice according to the conspecific's visual features (Kellogg 1997; Seehausen and van Alphen 1998; Carleton et al. 2000), social organization, and foraging behaviors in complex habitats (Dobberfuhl et al. 2005). In the present study, all individuals that reached the learning criterion maintained high levels of performance (bamboo sharks: 78.70% ± 0.09 correct decisions, Malawi cichlids: 82% ± 0.11 correct decisions) during the regular trials of the transfer phase. In particular, results of Training 4 suggest that both bamboo sharks and Malawi cichlids were able to combine at least two relational features from learned experience with familiar complex stimuli. If individuals had only learned to rely on two "item-specific features" (e.g., "avoid horizontal lines and approach cross patterns"), they would have had serious problems solving all training tasks as the stimuli in each of these tasks were different (Figure 2A). The individuals' experience with single objects possibly gained during previous experiments (cf. Fuss et al. 2017; Schluessel et al. 2018) would not have been sufficient to solve the present tasks.

In the present study, bamboo sharks and Malawi cichlids were trained to choose a stimulus comprising two different, vertically arranged objects and to neglect the horizontal alternative. Stimulus features and positions were systematically varied between training tasks (Figure 2A), maintaining only the above/below or left/right relationship as the only criterion of reward, while preserving the requirement that simultaneously presented stimuli contained two different objects (especially in the fourth training task; Figure 2A)— a training scheme also used for bees by Avarguès-Weber et al. (2012). During training, individuals learned to choose the rewarded spatial relationship between stimuli, regardless of the absolute position of stimuli in the individuals' visual field. However, both alternatives (i.e., the vertical and the horizontal option) comprised two different objects whose appearance (geometric patterns) were identical. Hence, one might argue that it would have been possible to solve the trainings tasks by recognising the "2D" stimulus as a single bar or rectangle, rather than a combination of two different squares without paying any attention as to whether or not the two objects

forming a stimulus were different. However, this does not explain the Malawi cichlids' serious problems to solve most of the training tasks. Similarly, considering the bamboo sharks' training performance, the number of training sessions to solve the task decreased from Training 1–3 in three out of six individuals. For the remaining three, the number of sessions clearly increased or they could not solve their task at all. In addition, the bamboo sharks' number of sessions to reach the learning criterion in the fourth training task remarkably increased again or remained at a similar level as before, possibly indicating the use of different strategies to solve the training tasks. If sharks or cichlids had relied on a simpler "vertical versus horizontal plain" strategy, they should have been able to easily solve at least training tasks 2–4 equally well (which does not apply to three sharks and five cichlids; Table 1) or maybe even spontaneously correct. In addition, in most transfer tests bamboo sharks showed that they took both features (i.e., spatial alignment and object's appearance) into account. Generally, bamboo sharks and Malawi cichlids are able to learn, through experience, to discriminate simple and complex objects based on coincident visual features (bamboo sharks: e.g., Fuss et al. 2014b, Fuss and Schluessel 2017; Malawi cichlids: e.g., Schluessel et al. 2012, 2014b), to master discriminations based on movement and symmetry perception (e.g., Schluessel et al. 2014b; Fuss et al. 2017) and even to navigate in complex mazes (e.g., Fuss et al. 2014c, 2014d). In these experiments, it has been frequently shown that bamboo sharks and Malawi cichlids recognized different aspects of both stimuli (positive and negative ones) and distinguished based on (small) details between both alternatives. Therefore, it seems reasonable to infer that bamboo sharks also applied this capability in the present study and did not predominantly rely on a single feature (e.g., a specific spatial alignment). Nevertheless, the adoption of a simple "vertical versus horizontal plain" strategy cannot be completely ruled out with regard to the results of most transfer test trials, in which the Malawi cichlids apparently chose indiscriminately.

Surprisingly, despite consistently high performances of all bamboo sharks during training and most transfer trials, only one shark succeeded in the T1 trials presenting novel gray and black circles. Additionally, one shark and one cichlid showed a robust but non-significant preference for the correct alternative (Shark 2, Cichlid 5). In both cases, individuals chose the appropriate spatial relationship in the respective transfer test in on average 10.4 ± 3.61 (i.e., 52%, bamboo sharks) to 13 ± 0.63 trials (i.e., 65%, Malawi cichlids). During Training 1, 2, and 3, the stimulus sets were not changed until the learning criterion was reached, allowing individuals to solve the task based on direct association between the food reward and each positive stimulus—a training scheme also used for archerfish by Newport et al. (2014, 2015). Yet, ten individuals of the present study presumably learned the general idea to "choose two different, vertically arranged objects" and were able to transfer this knowledge to the fourth training task (as discussed earlier). The bamboo sharks' weak and the Malawi cichlids' moderate performance in the T1 trials match these results, although they seem to pretermit the use of abstract rules or "concepts". Even though the same training stimulus sets were presented randomly on the right and left side of the screen, individuals quickly chose the correct alternative during regular training trials. It is therefore unlikely that the task was solved based on the presence or absence of discrete key parts of a stimulus.

The results of transfer tests T2 – T4 (gray and black circles), T5 – T6 (different squares with geometric patterns) and T9 (circles and bar) showed that the tested bamboo sharks can do much more than

just associate the visual properties of a target with a reward. When bamboo sharks were presented with conflicting information in these transfer tests performance remained at a high level, indicating the application of the relational aspects of the presented stimuli, i.e., the abstract concepts of "sameness" or "difference" and—to some extent—spatial arrangement ("vertical" or "horizontal"). In these transfer trials, individuals had to choose either between two different or two same objects aligned at different angles. Four out of five bamboo sharks preferred the "2D" to the "2S" stimuli both in combination with the appropriate (T2) and the inappropriate (T3) spatial relationship. The latter was also the case for three bamboo sharks, which chose "different" even when conflicting information was provided (although the correct spatial relationship was associated with two same objects, while the wrong spatial relationship was linked to the two different objects, T4). Similarly, all bamboo sharks significantly preferred the "2D" stimuli in T5 trials using squares with different geometric patterns. While three bamboo sharks significantly preferred the "2D" stimuli in T6 trials, two others showed a distinct preference for this stimulus. The same applied for two bamboo sharks in T7 trials (including three individuals showing a distinct preference for "2D" stimuli). Generally, all bamboo sharks significantly preferred the "2D" stimuli in transfer trials providing conflicting information with respect to appearance and spatial arrangement of the objects forming a stimulus. These results seem to confirm that four bamboo sharks learned to recognize the "different" objects as an important stimulus feature. The preference for "difference" over "sameness" was so strong that bamboo sharks chose the two different objects in T3/T4 and T6/T7 trials; although, the spatial orientation was explicitly associated with food deprivation and immediate return to the starting compartment during training. These results allow for different interpretations: (1) either "difference" was more important than the spatial relationship or (2) both rules were taken into account to varying degrees or (3) the meaning of the negative stimulus was not learned appropriately during training. With respect to the bamboo sharks' consistently high training performance, it is likely that they have combined both rules—although they seem to be of varying importance as revealed by the transfer tests. Bamboo sharks seemed to predominantly apply not only the rule of difference when judging both stimuli, but they also considered the arrangement in a specific spatial relationship to a lesser extent (as individuals would not have been able to succeed in training).

Conversely, Malawi cichlids mostly appeared to choose randomly in transfer tests using gray and black circles (T1 – T4) or squares with different geometric patterns (T5 – T7). In this case, the feature "difference" (or "sameness") may have been equally important to the feature "spatial relationship", which would have led to confusion when conflicting information was provided. This is supported by the inconsistent performance in the spatial versus geometry transfer trials (T5 – T7), i.e., while most individuals preferred the "2D" stimulus in T5, the same individuals chose indifferently between both alternatives or favoured the "2S" stimuli in T6 and T7 trials. Moreover, Malawi cichlids performed generally slower in comparison to regular training trials, indicating that they noticed that transfer stimuli differed from those used during training. These results are consistent with previous attempts to teach teleost fish different abstract concepts (Malawi cichlids: asymmetry/symmetry, Schluessel et al. 2014b, archerfish: sameness/difference, Newport et al. 2014, 2015).

Of course, the level of learning and the application of abstract rules or even concepts in fish could be fundamentally simpler compared to humans, mammals or birds. To give an example, humans can assess the ways in which sailboats, surfboards or paragliders are similar (as they all use the wind as a driving force) and in which ways they are different. But, in many tasks used to train non-human species, the presented stimuli are either similar or not. Basically, "this involves comparing an incoming stimulus with the contents of working memory, and the rule of whether to choose 'same' or 'different' [or to consider a certain spatial relationship] must be stored in long term memory—but it does not necessarily require the formation of a concept with the full range of implications of the term in human psychology" (Chittka and Jensen 2011). The question whether rule- or even concept-formation in fish can be explained by mechanisms such as stimulus generalisation (Shettleworth 2010; Zentall et al. 2008) and/or discrimination by indicative perceptual features ("first-order perceptual features", Chater and Heyes 1994; Tomasello and Call 1997) has to be considered when interpreting the present results. Accordingly, low-level explanations should be considered as potential interpretations of some transfer performances. One could discuss whether some objects in transfer tests using different squares (T5 – T7, T10) were reminiscent of training objects (e.g., vertical or horizontal lines or an altered cross), which individuals may have used as a kind of "template" when facing the transfer stimuli. However, although template matching can potentially account for the bamboo sharks' transfer performance in T7/T10, it can be ruled out for T5/T6, within which bamboo sharks chose against the predictions of a simple visual strategy. Therefore, the simultaneous mastering of two visual relational rules by bamboo sharks appeared to reflect a higher level of complexity than just peripheral image coincidence. Similarly, one could discuss whether bamboo sharks could have rapidly learned to attend to "odd features" which stand out visually (e.g., a gray circle or vertical/horizontal lines) in some transfer tests. Such higher-order rules would not solely depend on low-level feature analyses. This explanation also appears to be unlikely as transfer trials generally remained unrewarded and thus, this strategy would not have been beneficial (just as choosing the alternative confining two similar objects). Moreover, if bamboo sharks had initially applied this "odd one out"-strategy, it would have been likely that they had changed it during a series 20 transfer trials, at least one or the other individual. Likewise, their constantly high choice consistency in virtually all transfer tests appears to contradict this idea. However, these low-level explanations cannot be ruled out for Malawi cichlids.

All individuals were also examined for their potential reliance on object similarity, a proper relational alignment, or alternatively, other broader cues (e.g., global orientation of objects). The corresponding transfer tests (i.e., T2/T3, T5/T6, T8/T9, T10) tested learning and discrimination ability of (a) objects connected by a specific relationship (e.g., one above the other or one next to the other one) and (b) a global cue (e.g., a general vertical or horizontal arrangement) associated with food. Bamboo sharks preferred two different gray and black circles to a single gray bar if they were linked by the inappropriate horizontal relationship (T8/T9), and if they were displayed in the appropriate vertical relationship (T2/T5). This suggests that bamboo sharks did not use the global orientation of the gray and black circles as a lower-level cue to solve this new task. The presence of the two different gray and black circles appeared to be more important than one strong spatial cue (i.e., one vertical bar). Surprisingly, they relied on the same strategy when achromatic different squares were displayed (T5), but mostly a different strategy when approaching a squares versus a bar (T10). Malawi cichlids made their decision by chance when facing different circles (T8/T9), but significantly favored the "2D" objects when facing different squares (T10). The vertical

bar, despite representing a prominent spatial cue was not perceived as representative of the rewarded stimulus.

In conclusion, results indicate that bamboo sharks primarily learned to choose two different objects, and were able to apply this knowledge to transfer tests with different circles or squares (i.e., irrespective of objects used to build this relationship). However, results suggest that the level of learning and usage of both abstract rules or possibly even "concepts" differed between bamboo sharks and Malawi cichlids. Bamboo sharks seemed to combine both concepts while weighing them differently, thereby showing advanced cognitive prerequisites, independent of the perceptual nature of the presented stimuli. Irrespective of the objects' appearance, bamboo sharks (a) learned to choose objects arranged in a specific spatial relationship, (b) learned that all stimuli were composed of different visual elements, and (c) transferred this knowledge to a variety of transfer stimuli. It is likely that a dual concept based on two distinct relational rules rather than perceptual similarity guided the bamboo sharks' choices during training and most transfer tests. Results obtained from the bamboo shark experiment in the present study are in line with earlier categorization experiments using single abstract concepts on the same species and, to some extent, point to similar concept forming capabilities as found in harbour seals (Scholtyssek et al. 2013), echidna (Russell and Burke 2016), parrots (Pepperberg 1987), and pigeons (Katz and Wright 2006).

Conversely, Malawi cichlids failed to correctly apply training knowledge when presented with different sets of geometric patterns or, in particular, black and gray transfer stimuli. Therefore, learning mechanisms such as stimulus generalisation and/or discrimination by "first-order perceptual features" cannot be excluded. Unfortunately, it is impossible to determine a general strategy or one used by specific Malawi cichlids to solve the task, which could take on very different levels of complexity. Additionally, there may be intraspecific differences regarding the underlying mechanism, i.e., while some individuals may have solved the (training) task using simple cues (e.g., only focussing on "vertical" or "horizontal" arrangement), others may have used a more complex strategy (e.g., a rigorous combination of "vertical" and "two different objects").

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## Ethical statement

The research reported herein was performed under the guidelines established by the EU Directive 2010/63/EU for animal experiments and the current German animal protection law and had been approved by the Landesamt für Natur, Umwelt und Verbraucherschutz NRW (approval number 8.87-50.10.37.09.198).

## Supplementary Material

Supplementary material can be found at http://www.cz.oxfordjournals.org/.

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