

Social learning of escape routes in zebrafish and the stability of behavioural traditions

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Multiple factors potentially influence the formation and longevity of behavioural traditions. In zebrafish, *Danio rerio*, we investigated whether subjects follow knowledgeable fish escaping from a novel artificial predator, learn this escape response, and maintain the demonstrated escape route and response when knowledgeable fish were removed. A moving 'trawl' net forced fish to escape via one of two equidistant escape routes. Groups of four naïve fish were placed together with demonstrator fish trained to use either one of the two routes. Observers with demonstrators were faster to escape than observers exposed to untrained fish, and were biased towards the demonstrated route, effects that persisted when demonstrators were removed. Thus zebrafish socially learned escape routes and to escape faster from the approaching trawl. To address whether escape responses were stably transmitted, we used a transmission chain with observers becoming demonstrators for further groups of observers, thus simulating three generations of social learning. Escape times remained stable along the transmission chain, but route preferences slowly collapsed. Thus while the escape response per se was reliably socially transmitted, more arbitrary choices such as route choice decayed rapidly over repeated episodes of social learning. Our results suggest pervasive species and population differences in social-learning propensities. © 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Social learning is widespread in animals (Galef & Giraldeau 2001), and social information can be passed repeatedly from individual to individual leading to the formation of traditions, as demonstrated in both laboratory and field studies (Corten 2001; Brown & Laland 2003; Whiten & Mesoudi 2008). Factors that influence the stability of social traditions, such as group turnover, the costs and benefits of alternative actions and the possibility of individual exploration, are all known to affect tradition dynamics (Lefebvre 1986; Galef & Whiskin 1997; Stanley et al. 2008; Whiten & Mesoudi 2008). Strikingly, traditions can maintain arbitrary behaviour patterns or inhibit the acquisition of optimal behavioural patterns (Warner 1988; Galef & Whiskin 1997; Laland & Williams 1998; Bates & Chappell 2002; Reader et al. 2008; Stanley et al. 2008; but see Galef 1995, 1996). Thus there is a need to investigate the longevity of arbitrary versus nonarbitrary socially transmitted

traits (Thornton & Malapert 2009). Here, we studied the social learning of predator evasion to address these issues.

Grouping provides protection against predators (Pitcher & Parrish 1993; Roberts 1996). For instance, guppies, *Poecilia reticulata*, form larger, more cohesive shoals in areas containing adept guppy predators than in areas of decreased predation risk (Magurran 2005). Grouping tendencies and behavioural homogeneity within groups are important for minimizing predation risk: individuals that leave a group or behave differently from the group would be at increased risk (Landeau & Terborgh 1986; Theodorakis 1989), leading to the prediction that grouping individuals will follow the escape routes of others.

Individuals may gain further antipredator benefits from group members by using conspecific cues that indicate predator activity (social information use) and by learning about predators as a result of these cues (social learning). Such processes have been demonstrated in multiple taxa (Griffin 2004; Morand-Ferron et al., in press). For example, information on predators is socially transmitted in the European blackbird, *Turdus merula*, by observation of mobbing (Curio et al. 1978), in minnows, *Phoxinus phoxinus*, by observation of predator inspection behaviour (Pitcher et al. 1982; Magurran 1986), and in zebrafish, *Danio rerio*, by visual observation

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of alarmed conspecifics and by alarm substances passively released from injured skin (Hall & Suboski 1995a, b). Socially learned and socially facilitated antipredator responses to novel predator stimuli have been demonstrated in a number of shoaling fish species, as has social learning spanning several contexts and long-lasting behavioural traditions in the wild (Brown & Warburton 1999; Corten 2001; Brown & Laland 2003; Kelley et al. 2003). This leads to the prediction that when there is a cost to leaving the group, shoaling fish (and other grouping animals) will learn escape routes and escape responses from conspecifics, and that these escape responses will be stably transmitted to form behavioural traditions.

Studies of the social learning of escape routes provides a useful methodology to study tradition stability, but research on wild and domesticated guppies has produced apparently conflicting results (Brown & Laland 2002; Reader et al. 2003). In these studies, fish followed knowledgeable individuals. However, when these demonstrators were removed, wild but not domesticated guppies were biased towards their demonstrators' escape route. Genetic and experiential differences could account for these data, since laboratory guppies have not experienced predators within their lifetime or their recent evolutionary past. Guppies moved from a high- to a low-predation site show reduced shoaling tendencies (Magurran 2005), and thus it is likely that domesticated guppies show reduced antipredator behaviour and less cohesive shoaling compared to wild guppies, both of which could limit the learning of escape routes.

Group composition and group size may be important additional influences on shoaling tendencies and social learning. Fish make active choices about with whom to shoal, on the basis of characteristics such as group size, body size, familiarity and competitive ability (Krause et al. 2000), which may direct the flow of social information (Sih et al. 2009). Although enlarged groups may compromise foraging discoveries if fish need to break visual contact with the shoal to locate food (Lachlan et al. 1998; Day et al. 2001; Stanley et al. 2008), fish in larger shoals typically perform better in foraging and escape tasks (e.g. Pitcher et al. 1982; Brown & Warburton 1999). Moreover, numerous studies show the rate of social learning increases with the number of demonstrators in a group (Laland 2004).

We studied social learning of antipredator responses in zebrafish. Zebrafish development, genetics and neurobiology have been extensively studied, making them a potentially valuable but relatively unexplored species for behavioural studies (Gerlai 2003; Miklosi & Andrew 2006; Wright et al. 2006; Spence et al. 2008). We investigated whether zebrafish follow knowledgeable conspecifics trained to escape from a novel artificial predator (a moving 'trawl' net), whether they learn from this experience, and whether this behaviour is stably transmitted across generations. Fish could escape from an approaching trawl through one of two visually distinctive holes in an opaque partition. Both escape routes were equidistant and led to the same location and were hence functionally identical.

The experiment had three phases. In phase 1, 'demonstrators' were trained to use one of the escape routes. We counterbalanced demonstrator route training to account for the possibility that one route was intrinsically preferred. In phase 2, naïve fish ('observers') were placed with demonstrators while both routes were open for use. To follow social-learning terminology, we term subjects 'observers', but observers could both observe and interact with demonstrators. In phase 3, we tested observers without demonstrators to examine learning. We predicted that subjects would use the same escape route as the demonstrators and escape faster than fish without trained demonstrators. Following was predicted to result in learning about the escape response and route, with these behaviours maintained in the absence of demonstrators. Moreover,

we predicted improved social learning from increased numbers of knowledgeable conspecifics. We used demonstrators and subjects of two body size classes. Body mass can affect competitive ability and shoaling preferences in fish (Laland & Reader 1999; Krause et al. 2000), potentially influencing social learning (Duffy et al. 2009).

To address the stability of socially transmitted information we used a transmission chain design. Observers in one experimental treatment became demonstrators for a further set of observers, who then acted as demonstrators for another set of observers, thus simulating three generations of social learning.

METHODS

Subjects and Housing

A total of 300 female zebrafish (age 4–6 months) of the AB strain were used, originally obtained from the Max-Planck Institute, Tübingen, Germany, and bred and reared in the Biology aquarium at Utrecht University. We used a single sex to avoid sexual interactions during the experiment. The fish had not previously participated in any experiments. The experiment was approved by the Utrecht Ethics and Animal Care and Use Committee.

Fish were reared from birth in mixed-sex groups. Demonstrators and observers were reared and housed separately to avoid possible familiarity effects (Swaney et al. 2001). Two weeks before the experiment fish were moved to housing tanks in all-female groups. All housing tanks (80.0 × 50.0 cm) were maintained at 26 ± 1 °C, and had a water depth of 30 cm. Housing was enriched with artificial plants, pot shelters and gravel floor. Fish were on a 12:12 h light:dark schedule with lights on at 0800 hours (no natural light present). Fish were fed flake food (TetraMin, Tetra Ltd., Melle, Germany) twice daily, 1 h before and after experimental sessions.

We used fish differing in body size to allow discrimination of demonstrators and observers and to address any influence of body size, that is, large demonstrators were tested with small observers and vice versa. Sixty fish were chosen from rearing tanks at random to act as demonstrators, with equal numbers of large and small fish. We housed these fish in three tanks, each containing 20 individuals (10 large and 10 small). One tank was assigned to yellow route training ('yellow-trained demonstrators'), one to red route training ('red-trained demonstrators') and one to no training ('sham demonstrators'). Large and small demonstrators were separated from one another by a perforated transparent barrier that allowed movement of water but not fish. Demonstrators were reused during the experiment.

We used 240 fish (120 large and 120 small fish, mean mass \pm SE = 0.43 ± 0.01 g, 0.34 ± 0.01 g, respectively) as subjects (observers), including 80 that participated in the transmission chain. Observers only participated once in the experiment. Fish were tested in groups. Observers were always tested in groups of four, but demonstrator number varied with experimental treatment: (1) two demonstrators (treatment 2-DEMO, $N = 10$ groups of observers); (2) four demonstrators (treatment 4-DEMO, $N = 10$ groups); or (3) six demonstrators (treatment 6-DEMO, $N = 10$ groups). The control treatment used four (sham) demonstrators (Control, $N = 10$ groups). Thus 40 groups of observers (160 fish) were tested. The experiment was counterbalanced so that equal numbers of groups within each experimental treatment were exposed to red- and yellow-trained demonstrators (within the control treatment all demonstrators were sham demonstrators). There were also equal numbers of groups exposed to large and small demonstrators. The 4-DEMO treatment continued as a transmission chain, in which each of the 10 groups of observers became demonstrators for a group of four naïve observers, and then

these observer groups became demonstrators for another 10 groups of naïve observers.

Apparatus

Testing was conducted in a large tank (150 × 50 cm and 30 cm deep; Fig. 1) divided in half by a white opaque PVC partition with two square and visually distinct escape holes (1.5 × 4.0 cm each; placed at 15 cm from the bottom of the tank) 30 cm apart. In the 'trawl' zone the trawl net could be moved to within 2 cm of the partition, and the tank area was otherwise empty. The other, 'escape', zone was enriched with a gravel floor throughout and four randomly caught unfamiliar companion fish, plants and pot shelter were placed behind a transparent partition (with holes to allow for olfactory cues). This enrichment was provided in an attempt to make the escape zone the preferred zone for fish, and thus to minimize any swimming back and forth between compartments. To ease visual discrimination (Spence & Smith 2008; Spence et al. 2008), escape holes were bordered by electrical insulation tape: either 3 cm of red tape surrounded by a vertical 20 × 10 cm black/white striped area, or an identically sized yellow border surrounded by a horizontal black/white striped area. The trawl device (47.0 × 42.5 cm) was made of black mesh attached to a plastic-coated metal frame. Soft brushes attached to the side and base of the trawl prevented fish from escaping around its sides. The side of the tank facing the experimenter was covered with one-way glass.

Procedure

Each session consisted of four trials of 2 min each. During testing, fish were allowed 5 min to acclimatize after being moved between tanks and 1 min between trials. Fish were tested in two batches, with the schedule such that both demonstrator groups received the same number of days of training and the same interval between training and testing. Measurements were based on Sony DCR-SR55E video recordings.

Phase 1: demonstrator training

Fish were trained in groups of 10 to use either the red or the yellow route while the alternative route was blocked with transparent plastic. One training session consisted of four 2 min trials with 1 min rest between trials. Demonstrators received two training sessions a day. A trial began with the trawl moving towards the partition. It was moved back and forth four times in the 2 min,

and paused every 15 s at the tank end and at the partition. To begin training, enlarged escape holes extending to the bottom of the tank were used. When demonstrators did not exit through the escape hole, we used a brush to manoeuvre them to nearby the hole until they exited through it. When demonstrators were reliably swimming through the large hole in response to the trawl alone, this partition was replaced by the standard partition, with smaller escape holes. After a trial, fish that successfully escaped were gently herded back to the trawl zone by lifting the central partition. Demonstrators were considered fully trained when at least 80% escaped from the trawl within 30 s on four consecutive trials. Training continued for 2–3 days after demonstrators reached this criterion and then fish were given 3–4 days of rest. Control (sham) demonstrators were familiarized with the set-up for 2 consecutive days with two sessions per day, undergoing the same procedure as the other demonstrators except that the trawl was stationary and both holes were blocked by transparent sheets to prevent fish learning the escape routes.

Phase 2: testing demonstrators and observers

Depending on the experimental treatment, two, four or six (small or large) demonstrators were randomly selected from the trained demonstrator groups per session. Before we started the test session, demonstrators repeated one four-trial training session to confirm that they still used their trained route within 30 s. All demonstrators met this criterion. Four randomly chosen naïve observers of a different size class were then placed with the demonstrators. Fish experienced one session composed of four trials, similar to the training session, except that both escape routes were open. We recorded escape latency and escape route. If a fish did not escape from the trawl it was given a ceiling value of 2 min. Control fish underwent the same procedure but were placed with a group of sham demonstrators.

Phase 3: testing observers

Demonstrators were removed and observers were tested after a 5 min pause for their route preference and latency in one session with four trials. The procedure was otherwise identical to that in phase 2.

Transmission chain

The 4-DEMO treatment continued after completion of phases 1–3. Former observers acted as demonstrators for a new set of four naïve observers (as in phase 2, without the training session to check

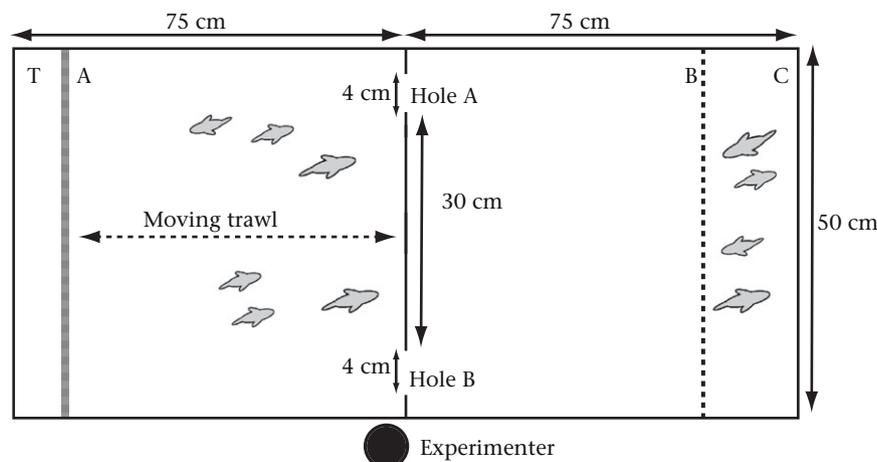


Figure 1. Schematic plan of the experimental tank. A: trawl zone; B: escape zone; C: companion fish; T: trawl device. Hole A: red escape hole; Hole B: yellow escape hole. The trawl was pulled back and forth in zone A towards a central white partition with two holes that allowed subjects to escape.

performance), and then the observers were tested alone (as in phase 3). Between sessions, new demonstrators were given a 5 min acclimatization period. These observers then acted as demonstrators for another set of four naïve observers, who were subsequently also tested alone. Thus, the 10 groups of 4-DEMO fish were further used for the transmission chain phase, resulting in 10 'chains' to which two groups of four fish were separately added. The transmission chain was counterbalanced such that there were equal numbers of groups that had experienced yellow and red route demonstrators, and equal numbers of groups of large and small body size.

Analyses

Dependent variables were escape latencies and escape route choices, calculated for demonstrators and observers separately. Latencies were individually measured, but means of the demonstrator and observer groups were used in the analyses, as zebrafish are schooling fish (Pitcher & Parrish 1993; Gerlai 2003) and thus individuals within a group could not be considered to behave independently. Route choice was measured as the relative preference for the yellow route, that is, the number of fish that used the yellow route minus the number that used the red route, divided by the total number of escapees (i.e. (observers using yellow–red)/all observers escaping). Values could range from –1 (all fish use the red route) to 0 (no preference) to 1 (all use the yellow route). If no fish escaped on a given trial, no route preference was calculated. We calculated route choice per group for each trial in a session.

Analyses were performed in R 2.6.2 (R Development Core Team, Vienna, Austria) and SPSS 16.0.2 (SPSS Inc., Chicago, IL, U.S.A.). For observer data, escape latency and escape route choice were analysed using linear mixed-effects models (LMM) with repeated measures (trial) and nested random effects (formula lme of package nlme; Pinheiro et al. 2008) to estimate *P* values and degrees of freedom. Fixed effects were the number of demonstrators (two, four or six), demonstrator route training (red, yellow), and demonstrator body size (large or small). Demonstrator escape latency was treated as a covariate in the transmission chain models addressing observer escape latency. Initially, all explanatory variables were entered into the models. Two-way interactions were investigated and terms were sequentially dropped until the minimal model contained only terms whose elimination would significantly reduce the explanatory power of the model. Nonsignificant ($P > 0.1$) interaction effects are not described in our results. Demonstrator size had no statistically significant effects and thus does not appear in the analyses below. We used separate analyses to make comparisons with control data. Demonstrator data were analysed using *t* tests, with latencies and route choice averaged across trials. Model assumptions were checked using box and qq-plots. Latency and route choice measures could be estimated by normal distributions.

RESULTS

Phase 2: Demonstrator Performance: Observers Present

Escape latency

All trained demonstrators met the criterion of escaping within 30 s when together with observers (mean escape latency \pm SE = 17.2 ± 1.8 s), whereas the sham demonstrators were significantly slower than the trained demonstrators (*t* test: $t_{38} = 17.42$, $P < 0.0002$; mean latency \pm SE = 97.5 ± 3.3 s). Yellow-trained demonstrators escaped faster than red-trained demonstrators, although not significantly so (*t* test: $t_{28} = 2.00$, $P = 0.06$; mean escape latencies \pm SE = 13.6 ± 1.5 and 20.6 ± 2.1 s, respectively; Fig. 2) and 2-DEMO demonstrators escaped significantly

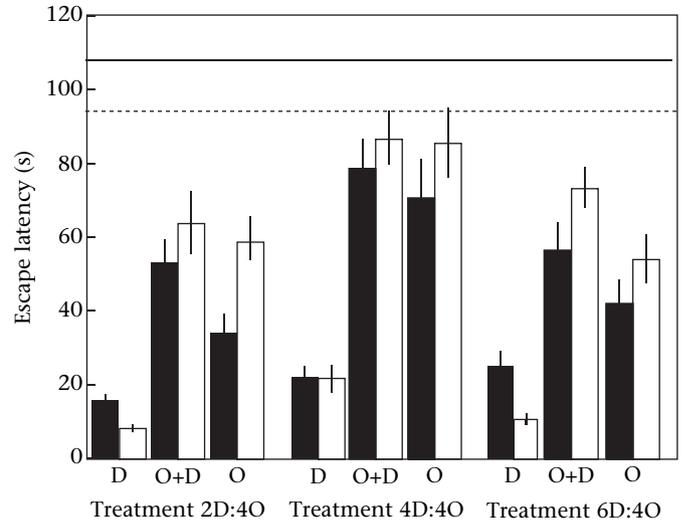


Figure 2. Mean escape latency \pm SE of groups of zebrafish demonstrators (D) and of observers in groups of four in the presence of two, four or six demonstrators (O + D), or after these demonstrators were removed (O). Demonstrators were trained to the red route (filled bars) or yellow route (open bars). Control ('sham') demonstrators were in groups of four. Control subjects' mean escape latencies with and without sham demonstrators are shown by the solid and broken horizontal lines, respectively.

faster than 4-DEMO demonstrators, but there were no other significant differences in escape latency between demonstrator groups of different size (*t* test: 2-DEMO versus 4-DEMO: $t_{18} = 2.59$, $P = 0.02$; 4-DEMO versus 6-DEMO: $t_{18} = 0.8$, $P = 0.4$; 2-DEMO versus 6-DEMO: $t_{18} = 1.93$, $P = 0.07$).

Route Choice

Red-trained and yellow-trained demonstrators escaped by their trained route on 93% and 96% of occasions, respectively (Fig. 3). On 75% of trials no sham demonstrators escaped, and when escaping they took the yellow route on 65% of occasions, suggesting a nonsignificant preference of naïve fish for the yellow route (mean route choice \pm SE value of sham demonstrators = 0.25 ± 0.2 ;

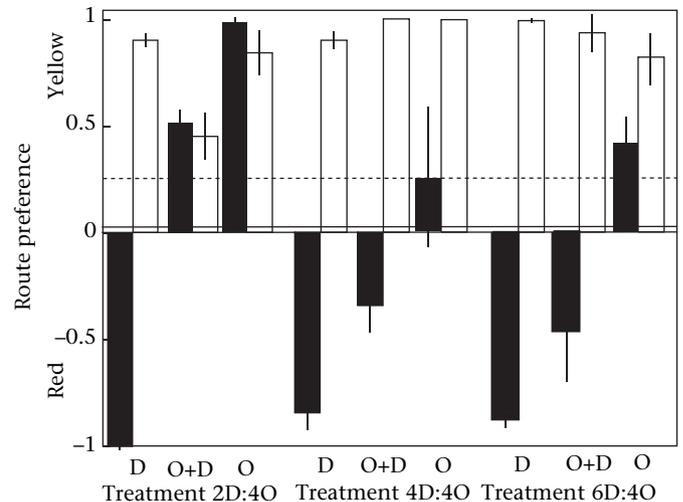


Figure 3. Mean route preferences \pm SE of demonstrators (D), of observers in groups of four with either two, four or six demonstrators present (O + D) and of observers after demonstrators were removed (O). Demonstrators were trained to the red route (filled bars) or yellow route (open bars). Sham demonstrators' and control observers' route preferences are shown by the broken and solid horizontal lines, respectively.

one-sample t test: $t_9 = 1.39$, $P = 0.2$). These results indicate that demonstrators performed according to their training, in terms of both their speed of escape and their route choice.

Phase 2: Observer Performance: Demonstrators Present

Escape latency

Observers escaped significantly faster with trained demonstrators than with sham demonstrators (mean \pm SE latencies = 69.4 ± 6.3 and 107.8 ± 3.5 s, respectively; t test: $t_{38} = 4.06$, $P = 0.0002$; Fig. 2).

Observers with red-trained demonstrators escaped faster than those with yellow-trained demonstrators (LMM: $F_{1,59} = 4.31$, $P = 0.04$; Fig. 2). The escape latencies of observers with trained demonstrators differed over trials (LMM: $F_{1,8} = 5.20$, $P = 0.05$), with observers escaping more rapidly on later trials (mean escape latency \pm SE = 87.9 ± 7.8 and 70.2 ± 7.6 s for trials 1 and 4, respectively). The number of demonstrators present did not significantly influence observer escape latency (LMM: $F_{1,1} = 0.97$, $P = 0.5$).

Route preference

Demonstrator route training significantly influenced observer route choice (LMM: $F_{1,57} = 21.35$, $P < 0.0001$): observers with red-trained demonstrators used the red route significantly more than those with yellow-trained demonstrators (mean route choice \pm SE = 0.03 ± 0.1 and 0.62 ± 0.1 , respectively; Fig. 3). Thus, demonstrator route training biased observers towards the route the demonstrators took. The interaction effect between the number of trained demonstrators and demonstrator route training approached statistical significance (LMM: $F_{1,57} = 3.40$, $P = 0.07$), and so we investigated this further. Demonstrator route training had a significant effect on observer route in groups with six demonstrators present, approached statistical significance in groups with four demonstrators, but was not statistically significant in groups with two demonstrators (t test: 6-DEMO: $t_8 = 4.78$, $P = 0.001$; 4-DEMO: $t_8 = 2.17$, $P = 0.06$; 2-DEMO: $t_8 = 0.69$, $P = 0.5$). Thus, observers were more likely to be biased towards the demonstrated route when more demonstrators were present. In the control condition, there was no significant difference in route choice between control observers and their sham demonstrators (paired t test: $t_9 = 1.12$, $P = 0.3$; mean route choice \pm SE observers and sham demonstrators = 0.05 ± 0.1 and 0.25 ± 0.2 , respectively).

Phase 3: Observer Performance: Demonstrators Absent

Escape latency

When demonstrators were absent, observers that had been paired with trained demonstrators escaped more rapidly than observers that had been paired with sham demonstrators (t test: $t_{38} = 2.96$, $P = 0.005$; mean escape latencies = 59.2 ± 6.3 and 93.7 ± 5.0 s, respectively). Observers that had been exposed to red-trained demonstrators escaped faster than those exposed to yellow-trained demonstrators (LMM: $F_{1,59} = 11.97$, $P = 0.001$; Fig. 2). The number of demonstrators did not significantly influence escape latency (LMM: $F_{1,1} = 5.04$, $P = 0.3$).

Observer groups that had experienced trained demonstrators escaped faster in the demonstrator-absent phase than the demonstrator-present phase (LMM: $F_{1,178} = 6.22$, $P = 0.01$; mean \pm SE escape latencies = 59.23 ± 6.3 and 69.42 ± 5.2 s respectively).

Route preference

Observers' route choices were significantly influenced by the route training the demonstrators had received (LMM: $F_{1,59} = 6.55$, $P = 0.01$): former observers of red-trained demonstrators used the yellow route significantly less than former observers of

yellow-trained demonstrators (mean route choice \pm SE values = 0.53 ± 0.1 and 0.75 ± 0.1 , respectively; Fig. 3). Thus, despite an apparent general bias for the yellow route, observers were biased towards the route the demonstrators had taken. The number of trained demonstrators did not have a significant effect on observer route choices (LMM: $F_{1,1} = 0.20$, $P = 0.7$). Control observers' route use did not differ significantly from their sham demonstrators' route use (paired t test: $t_9 = 0.40$, $P = 0.7$; mean route choice \pm SE values = 0.15 ± 0.2 and 0.25 ± 0.2 , respectively).

Observers that experienced red route demonstrators used the red route more with than without demonstrators (t test: $t_{178} = 14.35$, $P < 0.0002$; mean route choice \pm SE = 0.03 ± 0.1 and 0.53 ± 0.1 , respectively). In contrast, route choice of observers of yellow route demonstrators did not change significantly when demonstrators were removed (t test: $t_{28} = 0.86$, $P = 0.4$). Similarly, route choices of observers of sham demonstrators did not change significantly when these sham demonstrators were removed (paired t test: $t_9 = 0.80$, $P = 0.5$; mean observer route choice \pm SE values = 0.05 ± 0.1 and 0.15 ± 0.2 , with and without demonstrators, respectively).

Transmission Chain: Demonstrator Performance: Observers Present

Escape latency

Demonstrators became slower to escape from the first to the second and third generations (Fig. 4; note that second-generation demonstrators were observers of first-generation observers, and third-generation demonstrators observed second-generation demonstrators). Demonstrators escaped faster than observers, but only in generation 1 was this effect significant (t tests: generation 1: $t_{18} = 5.33$, $P < 0.0001$; generation 2: $t_{18} = 0.87$, $P = 0.4$; generation 3: $t_{18} = 0.77$, $P = 0.5$). Red- or yellow-trained demonstrators did not differ significantly in escape latency (t test: generation 1: $t_8 = 0.09$, $P = 0.9$; generation 2: $t_8 = 1.97$, $P = 0.09$; generation 3: $t_8 = 0.28$, $P = 0.2$; Fig. 4).

Route preference

Demonstrator groups in chains seeded by red- or yellow-trained demonstrators differed significantly in their route preferences in

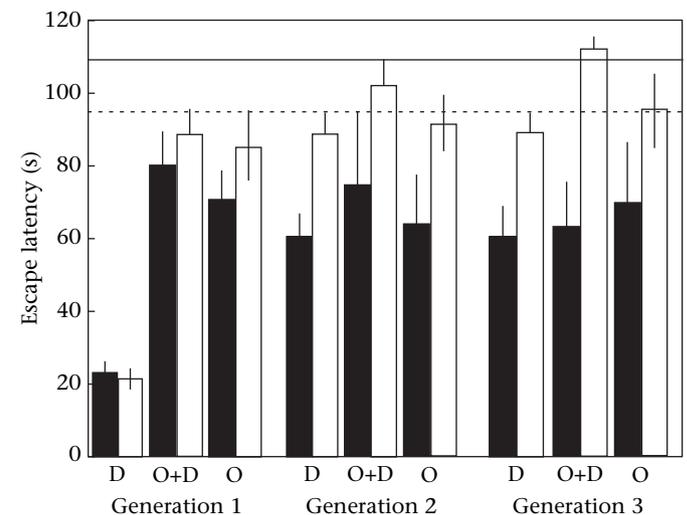


Figure 4. Mean escape latency \pm SE of demonstrators (D), observers with demonstrators present (O + D) and observers after demonstrators were removed (O) for each simulated generation in a transmission chain. Generation 1 demonstrators were trained to either the red route (filled bars) or yellow route (open bars). Observers of generation 1 demonstrators became the demonstrators of generation 2, and observers of generation 2 became the demonstrators of generation 3. Control observers' mean escape latencies with and without sham demonstrators are shown by the solid and broken horizontal lines, respectively.

generation 1 but not in generations 2 and 3 (t tests: generation 1: $t_8 = 9.96, P < 0.0001$; generation 2: $t_8 = 0.25, P = 0.8$; generation 3: $t_8 = 0.82, P = 0.4$). Only in generation 1 were route preferences biased towards the trained route. Red-trained demonstrators showed a preference for the red route and yellow-trained demonstrators a preference for the yellow route in generation 1, but in generations 2 and 3 route preferences were approximately equal (Fig. 5).

Transmission Chain: Observer Performance: Demonstrators Present

Escape latency

Demonstrator escape latency significantly influenced observer escape latency (LMM: $F_{1,56} = 45.43, P < 0.0001$): the faster demonstrators escaped the faster observers escaped. However, observer escape latency did not change significantly over generations (generations 1–2: t test: $t_{18} = 0.39, P = 0.7$; generations 2–3: t test: $t_{18} = 0.07, P = 0.9$). There was a significant interaction effect between generation and demonstrator route training on observer escape latency (LMM: $F_{1,56} = 3.87, P = 0.05$): observers in red-demonstrator-seeded chains escaped faster than those in yellow-demonstrator-seeded chains in generation 3 ($F_{1,8} = 12.09, P = 0.008$), while their performance was not significantly different in generations 1 and 2 (Fig. 4). In generation 3, observers from red-but not yellow-seeded chains escaped significantly faster than control observers (red versus control: $F_{1,13} = 13.38, P = 0.003$; yellow versus control: $F_{1,13} = 0.63, P = 0.44$).

Route preference

Over generations, observers with red-trained demonstrators increasingly used the yellow route to escape, whereas observers with yellow-trained demonstrators preferred the yellow route consistently over generations (i.e. there was an interaction effect between generation and demonstrator route training on route preference; LMM: $F_{1,19} = 10.17, P = 0.005$). The effect of demonstrator route training on route preference thus differed over generations, the effect being significant in generation 1 only (t test: generation 1: $t_7 = 3.47, P = 0.01$; generation 2: $t_7 = 0.31, P = 0.8$;

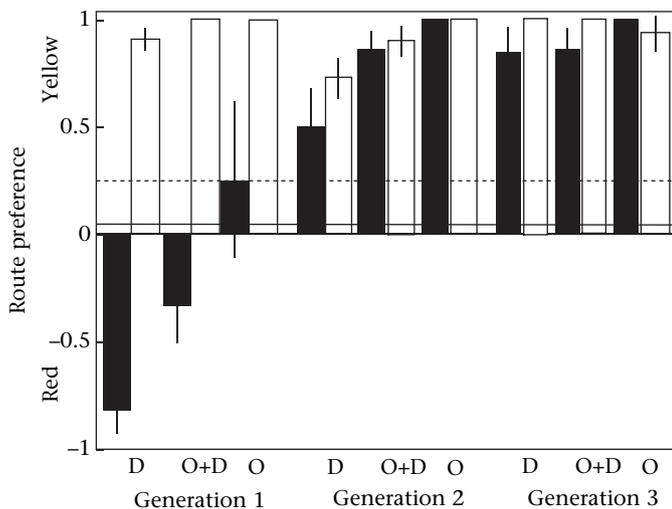


Figure 5. Mean route preferences \pm SE of demonstrators (D), observers with demonstrators present (O + D) and observers after demonstrators were removed (O) for each simulated generation of a transmission chain. Generation 1 demonstrators were trained to either the red route (filled bars) or yellow route (open bars). Observers of generation 1 demonstrators became the demonstrators of generation 2, and observers of generation 2 became the demonstrators of generation 3. Control observers' mean route preferences with and without sham demonstrators are shown by the solid and broken horizontal lines, respectively.

generation 3: $t_6 = 0.75, P = 0.5$; Fig. 5). Thus in generations 2 and 3 observers in red-seeded chains used the yellow route similarly to those in yellow-seeded chains, the opposite to their route preferences in the first generation.

Transmission Chain: Observer Performance: Demonstrators Absent

Escape latency

In the absence of demonstrators, former observers of red-trained demonstrators escaped faster than former observers of yellow-trained demonstrators (LMM: $F_{1,58} = 13.27, P < 0.0001$; Fig. 4). Observers were slower when demonstrators were present than when they were absent, but not significantly so (LMM: $F_{1,175} = 3.34, P = 0.07$). In generation 3, observers from red- but not yellow-seeded chains escaped significantly faster than control observers (red versus control: $F_{1,11} = 5.15, P = 0.044$; yellow versus control: $F_{1,11} = 0.05, P = 0.83$).

Route preference

As when demonstrators were present, there was an interaction effect between generation and demonstrator route training on route preference (LMM: $F_{1,98} = 24.57, P < 0.0001$; Fig. 5): observers in chains seeded with red-trained demonstrators increasingly preferred to use the yellow route over generations, while in yellow-seeded chains observers' preferences for the yellow route remained stable. The effect of demonstrator route training thus differed over generations, significant in generation 1 only (LMM: generation 1: $F_{1,7} = 9.05, P = 0.02$; generation 2: $P = 1.0$; generation 3: $F_{1,6} = 2.38, P = 0.2$). Observers did not show a significantly different route preference when demonstrators were present compared to absent, over all generations (LMM: $F_{1,98} = 2.77, P = 0.09$).

DISCUSSION

Naïve zebrafish with trained conspecific demonstrators escaped more rapidly from an approaching trawl than fish with untrained demonstrators. Moreover, demonstrator route training biased observers' route choices, a bias maintained when demonstrators were removed, results consistent with the observers learning an escape route from the trained demonstrators. Contrary to a recent social foraging study in stickleback, *Pungitius pungitius*, social foraging (Duffy et al. 2009), we did not find body size had a significant influence on social learning. In the transmission chain, observers at the end of the chain escaped faster than control fish, demonstrating that the escape response was transmitted across generations. However, route choice decayed rapidly over generations. Thus, arbitrary information such as route use was not stably transmitted.

Zebrafish probably learned both to escape and a specific route by following demonstrators that took only one route, increasing exposure to that route. Alternatively or in addition, demonstrators could have drawn more attention to one route (Swaney et al. 2001). Observational conditioning could also account for our results (Heyes 1994): observers could have learned to fear the trawl from visual, movement and/or olfactory demonstrator stress cues (Suboski et al. 1990), leading to more rapid escape, and, if demonstrators expose the observers to the relationship between one escape hole and the preferred 'escape' zone, to learning a particular route (Hoppitt & Laland 2008). Thus zebrafish could have learned by a number of nonmutually exclusive social-learning processes (Laland & Williams 1997; Boogert et al. 2008; Hoppitt & Laland 2008). In addition, social facilitation is likely to have accelerated escape latency in the demonstrator-present phase, although cannot account for continued rapid escape after demonstrators were removed. The decrease in observer latencies over trials when

demonstrators were present suggests that subjects were also learning asocially how to escape, but that trained demonstrators accelerated learning and biased learning towards particular routes.

The number of demonstrators had contrary effects on observer escape latencies and routes. We found strongest route following when larger demonstrator groups were present, and a similar (albeit nonsignificant) pattern when demonstrators were removed. In contrast, observers paired with two or six demonstrators tended to escape faster than those paired with four demonstrators in both demonstrator-present and demonstrator-absent phases, although these differences were not statistically significant. This pattern contrasts with the positive relationship between the number of demonstrators and acquisition described in guppies, rainbowfish, *Melanotaenia eachamensis*, rats, *Rattus norvegicus*, and pigeons, *Columba livia* (Lefebvre & Giraldeau 1994; Brown & Warburton 1999; Laland 2004). However, in line with our findings, zebrafish learned shock avoidance faster in groups of five or one compared to two, and Arctic char, *Salvelinus alpinus*, learned superior predator avoidance with fewer demonstrators per observer (Gleason et al. 1977; Vilhunen et al. 2005). Opposing processes may shape the influence of group size on social learning. For example, the larger the shoal the stronger its social attractiveness is (Day et al. 2001), promoting learning, while fear may decrease with increasing shoal size, thus hindering learning of escape responses. Relations between demonstrator number and social learning may differ between behavioural contexts and be nonlinear.

In common with our findings, both rainbowfish and guppies socially learn to escape faster from a moving trawl (Brown & Warburton 1999) and copy the route used by their demonstrators (Brown & Laland 2002; Reader et al. 2003). The social learning of route preferences we have demonstrated in zebrafish matches findings in wild guppies tested in the field (Reader et al. 2003), but not in domesticated guppies tested in the laboratory, which escaped faster after pairing with trained demonstrators but did not show a learned preference for a particular route (Brown & Laland 2002). Minor methodological differences could account for these results, such as the fact that fish escaped through a partition in the present study and in Reader et al.'s (2003) study, but through the trawl in the study of Brown & Laland (2002). Also, Brown & Laland (2002) tested observers several hours after the removal of demonstrators, another potential explanation for the loss of route preferences. Our study used a short (5 min) gap between training and test, although demonstrators were shown to maintain their training after 3–4 days without reinforcement. We argue that strain and species differences provide the most likely explanation for the differences between studies. Tighter shoals are formed by wild strains of both guppies (Kelley et al. 2003) and zebrafish (Wright et al. 2006), probably reflecting relaxed selection or developmental influences in domestic environments. In wild populations, the costs of leaving the shoal and engaging in individual exploration are more likely to outweigh the possible benefits. Zebrafish also form more polarized, tighter shoals than guppies (Suboski et al. 1990), and hence social learning by following may be stronger in the former. Environmental factors are tightly linked to grouping tendencies and the costs and benefits of differing from the group, potentially driving differences in social-learning propensities. Our results add to findings indicating that individuals, populations and species differ in their propensity to learn from one another (Lefebvre et al. 1996; Carlier & Lefebvre 1997; Reader & Laland 2002; Bond et al. 2003; Reader 2004; Kendal et al. 2005). The mechanisms that underlie such differences in social learning and their consequences for animal ecology and theoretical models of social learning are likely to be substantial but remain relatively unexplored (Reader 2004; Morand-Ferron et al., in press).

The transmission chain demonstrated that social information about escape behaviour is transmitted over multiple generations, a finding complementing previous studies on foraging (Laland & Williams 1997; Stanley et al. 2008). Subjects escaped more rapidly than controls, even at the end of the transmission chain (generation 3). However, this finding was restricted to fish in red-seeded chains, perhaps because of unusually poor yellow demonstrators at the beginning of generation 2. Fish from red-seeded chains increasingly took the yellow route over repeated episodes of transmission, resulting in equally strong preferences for yellow route use for all fish in generations 2 and 3. The two routes probably differed in attractiveness, resulting in a bias for the yellow route. At the end of the transmission chain fish had a stronger yellow route preference than control observers, an observation most probably explained by demonstration differences. Observers of sham demonstrators rarely escaped, and, when they did, escaped as individuals rather than taking one route together. In contrast, demonstrators in generations 2 and 3 provided a usable 'tip-off' as to how to escape, but not for the red route. This, combined with a slight bias for the yellow route of naïve fish, resulted in generation 2 and 3 observers escaping rapidly in coherent shoals via the yellow route.

Our finding that traditions collapsed when based on arguably relatively arbitrary information, such as a particular route when both routes are functionally identical, while the more functional aspect of escaping faster was still preserved along the chain, complements results in guppies (Laland & Williams 1997) and wild meerkats, *Suricata suricatta* (Thornton & Malapert 2009). However, the collapse of route preferences contrasts with the extremely stable traditions found in mate choice and foraging contexts in wild fish (Helfman & Schultz 1984; Warner 1988). This apparent disparity could be explained by observers not experiencing differential feedback (or costs) (Hoppitt & Laland 2008) when they used the alternative escape route: both routes led to the same location. In the Warner (1988) study, mating sites were arbitrary but an individual choosing an alternative site would have lost mating opportunities: there was a cost to being different from the group. Manipulation of the perceived costs and benefits of the two routes would be predicted to affect tradition stability. It is also possible that more salient landmarks would increase transmission stability. However, the fact that demonstrators remembered their route preference after a 3–4-day delay suggests that the opportunity for exploration of alternative equidistant routes is responsible for the collapse in the route tradition (Galef & Whiskin 1997).

Here, we attempted to mimic a realistic antipredator situation with a simulated predator approach and a transmission chain design, that is, by creating a founder population of demonstrators representing experienced individuals, and replacing this group repeatedly, representing an influx of naïve individuals. It could be argued that the fish learned not antipredator responses but instead routes through their habitat or to locate companions. However, subjects showed typical zebrafish alarm responses (Rehnberg & Smith 1988), and thus were learning when alarmed. Furthermore, nets and other fishing gear evoke predator escape responses in fish, and large moving objects are perceived as threat stimuli (Brown & Warburton 1997, 1999). Although zebrafish may react differently under natural circumstances with real predators, our experiment can be considered a predation test, and further investigation would indicate whether identical results would be observed in other situations or contexts. Recently, Stanley et al. (2008) argued that acquisition of tasks difficult to learn asocially (e.g. a rare innovation) provides the strongest evidence for social learning. Our task fails this criterion: control subjects did not readily escape, but would be expected eventually to learn the task individually. Acquiring rare innovations from others might provide compelling

evidence for social learning. However, situations where knowledgeable conspecifics accelerate learning that could occur asocially are likely to be extremely common and relevant to fitness, and thus warrant study.

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