Biol Invasions (2020) 22:3357–3369
https://doi.org/10.1007/s10530-020-02329-y

ORIGINAL PAPER

Behaviourally-mediated learning ability in an invasive marine fish

Emma M. DeRoy · Nigel E. Hussey · Hugh J. MacIsaac

Received: 28 June 2019 / Accepted: 29 July 2020 / Published online: 10 August 2020
© Springer Nature Switzerland AG 2020

Abstract Invasive species can have profound impacts in non-native environments, the mechanisms behind which are often unclear. Learning and memory are notably two traits that may facilitate their impact. Behavioural traits can subsequently mediate learning ability in invasive species, the interaction between which may provide means by which to both better understand and manage invaders. We evaluated this relationship in lionfish (*Pterois volitans*), a species introduced to and invasive in the western Atlantic Ocean. We trained lionfish in a food reward task and assessed the degree to which behavioural traits and navigation strategy influenced their performance. We then evaluated memory retention by subjecting fish to training breaks of 5 to 42 days. Lionfish exhibited high inter-individual variability in learning. Half of the lionfish tested learned to navigate the maze, whose performance was strongly mediated by behaviour. Learning ability was positively correlated with boldness, exploratory tendency, and speed of task completion, but irrespective of spatial navigation strategy. However, fast exploratory fish trained in the complex navigation strategy had difficulty adapting to changing environmental conditions, indicative of a speed-accuracy trade-off. Lionfish were able to remember the location of the food reward for up to 6 weeks. Behavioural-mediated learning may help explain and understand the high impact of lionfish and other non-indigenous species in their invaded range and may elucidate spatiotemporal context-dependencies in their ecological impact.

Keywords Behaviour · Invasive species · Impact · Behavioural syndrome · Speed-accuracy trade-off · Memory · Learning · Context-dependent · Lionfish

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-020-02329-y) contains supplementary material, which is available to authorized users.

E. M. DeRoy (✉) · H. J. MacIsaac
Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada
e-mail: deroye@uwindsor.ca

N. E. Hussey
Department of Biology, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada

H. J. MacIsaac
School of Ecology and Environmental Science, Yunnan University, Kunming, China

Introduction

The breadth of invasive species impacts is tremendous, in both scale and scope, ranging from those at the individual-level to population, community and ecosystem levels. A comprehensive understanding of the mechanisms underpinning ecological impacts is

needed to efficiently manage invasive species (Pyšek and Richardson 2010). To this end, considerable research has investigated the causes and correlates of invasion success. Behavioural traits and behavioural plasticity (i.e. learning and memory) are increasingly recognized as mechanisms important to invader impacts (Holway and Suarez 1999; Weis 2010).

Within a species, behavioural traits are likely to differ among conspecifics. Understanding how these traits vary among individuals in a population can be used to better understand invasion dynamics (Pintor et al. 2009). Accruing evidence links certain suites of behaviours (i.e. behavioural syndromes) possessed by invasive species to high impacts. For example, boldness and neophilia have been correlated with competitive superiority in invaders (reviewed in Pintor et al. 2009). While inter-individual variation in behaviour has generated copious studies, only recently have researchers assessed its effect on learning. Learning is required of animals to respond to environmental change (Brown 2012). The efficiency with which organisms learn and forage may portend long-term fitness consequences (reviewed in Dukas 2004). For example, learning speed has been correlated with foraging success (Raine and Chittka 2008).

Recently, researchers have suggested that intraspecific variation in behaviour may influence learning ability and vice versa (reviewed in Sih and Del Giudice 2012). This theory suggests that animals adopt a speed-accuracy trade-off, whereby faster and more exploratory individuals learn more quickly but may be less flexible because they attend to their environment less. Conversely, slower individuals may spend more time attending to environmental cues, make choices more accurately, and may be more responsive to environmental changes (Sih and Del Giudice 2012). How this relationship manifests in invasive species is presently unclear, as few studies have been conducted to examine it (but see Chung et al. 2017). Understanding this dynamic may help to understand the potential fitness consequences and invasion dynamics associated with behaviourally-mediated learning ability in invasive species. In this paper, we test whether variation in learning ability—and, hence, foraging success—can be explained by behavioural variation in an invasive marine species, lionfish (*Pterois volitans*). To our knowledge, no previous study has quantified personality-dependent learning in an invasive fish under controlled conditions.

Lionfish are native to the Indo-Pacific region but were introduced to Florida's east coast in the 1980s, following which it spread widely throughout the western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico (Schofield 2010). In the eastern Gulf of Mexico, a more recently invaded site by lionfish, resources are heterogeneously distributed amongst artificial reefs. Similar to other fishes that inhabit complex environments with patchily distributed resources, success of lionfish may depend upon determining and remembering the relative profitability of food patches (Hughes et al. 1992). How quickly they can learn to find food and how long they can retain those memories may affect their foraging success. Fish with better memories can develop superior frames of reference concerning patch profitability (Warburton 2003), and may deprive competitors of resources by depleting patches first (Schoener 1983).

Lionfish have exerted considerable ecological impacts in its invaded range (e.g. Albins 2013). Understanding how their behaviour and learning ability interact may help to inform behavioural traits associated with highly impactful invasive fishes including lionfish. Spatial learning is one method to investigate the relationship between behaviour and learning (e.g. Brydges et al. 2008). Using a food-reward task, we investigated whether spatial navigation strategy (simple vs. complex) and behavioural traits (boldness, exploratory tendency, and speed) influenced learning rate and ability in lionfish. These traits have previously been correlated with invasion success (Chapple et al. 2012). We then assessed retention of these food-related memories. We predicted that faster, bolder, and more exploratory fish would learn the task more quickly relative to slower, shyer, more neophobic fish. We did not expect that learning ability or memory duration would differ between complex and simple spatial navigation groups given the relevance of a food reward. Finally, we predicted that faster and more exploratory fish would take longer to learn the task when trained in the complex spatial navigation strategy, as it required fish to attend to their environment (see Sih and Del Giudice 2012).

Methods

Lionfish (N = 20, 216.53 mm TL ± 5.36) were collected during summer and fall 2018 off the coast of Bonita Springs, Florida. All fish were captured from a single sinkhole (26-28.900°, 82-44.190°) to eliminate the confounding influence of habitat type and complexity on learning speed and efficiency (White and Brown 2014), at an average of 30 m depth. Experimental fish were acclimated for a minimum 2 weeks prior to being used in experimental trials [fed *ab libitum* silversides (*Atheriniformes* spp.), pilchards (*Harengula jaguana*), and pink shrimp (*Penaeus duorarum*)] and were housed with conspecifics throughout the experiment. All animals were experimentally naïve. During the acclimation period, lionfish were tagged with Floy® FTSL-73 polyethylene streamer tags (4″ length), each containing a unique three-digit number. Live pink shrimp ($\bar{x}$ = 30 mm) purchased from a local bait shop served as prey for lionfish and were readily consumed.

Fish were housed in several 1135 L recirculating tanks in a semi-enclosed aquaculture cage equipped with chemical, physical, and biological filtration systems and subject to a natural photoperiod. In both housing and experimental tanks, water was maintained at 24 °C, pH 8.2, 34 ppt salinity, and dissolved oxygen (DO) at or near saturation. Ammonia, nitrate, and nitrite were monitored and never exceeded negligible levels.

Experimental protocol

Foraging in fish is guided by spatial navigation and underpinned by different mechanisms of spatial orientation. They can use an allocentric strategy to orient based on the relative position of environmental cues. This allows animals to find a location from a novel starting point (O'Keefe and Nadel 1978; Rodriguez et al. 1994). Alternatively, fish may orient using body-centric cues by turning in specific directions, termed an egocentric strategy. Lastly, they can use an ego-allocentric strategy by employing a combination of both aforementioned strategies (O'Keefe and Nadel 1978; Rodriguez et al. 1994; van Gerven et al. 2012). For ease, we will hereafter refer to each of allocentric and ego-allocentric strategies as *complex* and *simple* strategies, respectively.

We assessed the ability of lionfish to learn a food-reward task using either a: (1) complex strategy; or (2) simple strategy (Fig. 1). Lionfish trained using the complex strategy were required to orient using extra-maze cues to retrieve a shrimp prey item as opposed to their relative position in space. They could not complete the task by turning left or right. Lionfish using the simple strategy were trained to navigate the maze by turning either right or left. In this way, the complex strategy required lionfish to attend to their environment to successfully complete the task. Consequently, we predicted that bold lionfish would have difficulty learning the complex strategy, given that attention is incompatible with speed under the speed-accuracy trade-off hypothesis.

Lionfish were randomly assigned to either the complex or simple group (N = 10 per group) using the RAND function in Excel. Given that there were no significant differences in size and thus age (Wilcoxon rank-sum test: W = 26.5, $P > 0.10$), we did not expect any confounding influences of age on learning ability. Each of the two experimental groups was broken down into two sub-groups (N = 5 each) to control for the influence of inherent directional or location biases (per Rodriguez et al. 1994; White and Brown 2014). In the simple group, half of the fish (N = 5) were trained to turn left and the other half (N = 5) were trained to turn right, while in the complex group, half of the fish (N = 5) were trained to locate prey in G1 while the other half (N = 5) were trained to locate prey in G2 (Fig. 1).

The experiment was comprised of four phases: acclimatization, pre-training, training, and memory retention, throughout which we also scored lionfish behaviour. Fish were tested individually to eliminate the potentially confounding influence of social cues (Ingraham et al. 2016). Trials were conducted over several months, which allowed us to explore individual consistency in fish learning ability and behaviour. For all trial phases, fish were starved 72 h prior to experiments to increase the likelihood of responding to resource-related cues (Bell 1991).

Behavioural measurements

We quantified the following variables to assess lionfish learning ability and behaviour: (1) the order in which fish entered individual maze arms; (2) whether the first arm chosen was the goal arm (binary

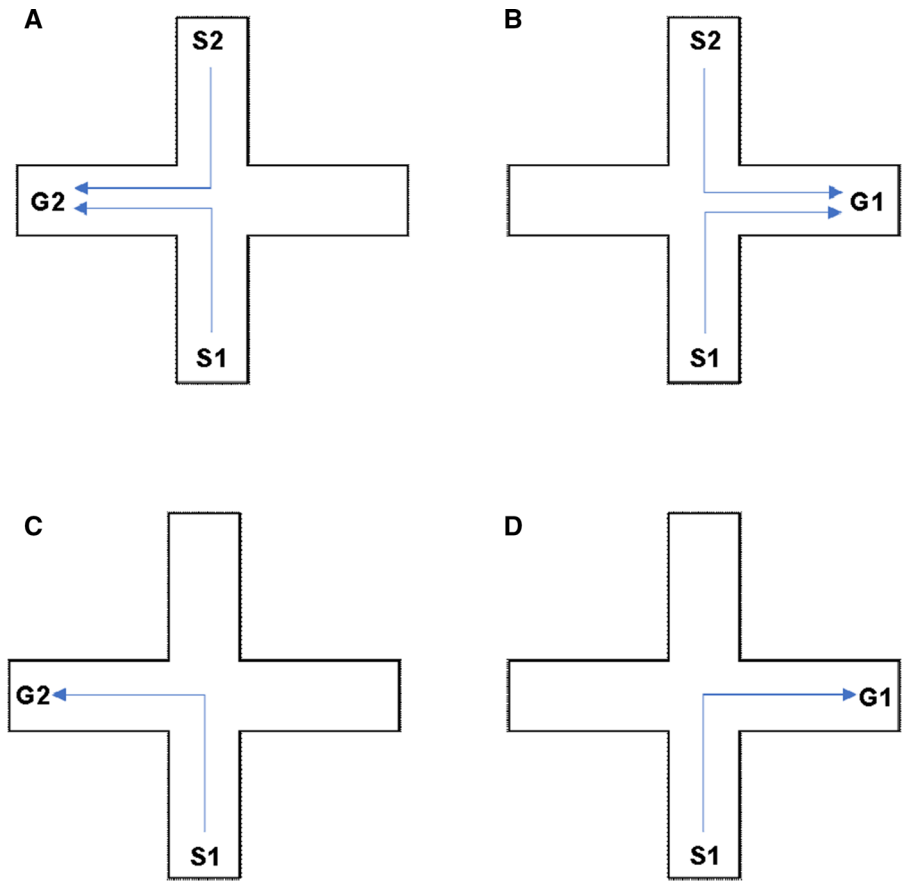

Fig. 1 **A**, **B** Complex strategy. Fish alternated at random from two start arms, S1 and S2. G1 and G2 refer to the alternative goal arms. Half of the fish in the complex group were trained to locate the shrimp in G2 (**A**), while the other half of the fish were trained to locate the shrimp in G1 (**B**). **C**, **D** Simple strategy. All fish consistently started from S1. Half of the fish in the group were trained to locate the shrimp in G2 (turn left) (**C**); the other half were trained to locate the shrimp in G1 (turn right) (**D**)

1/0, for goal arm or 'other'); (3) trial duration (time until task completion); (4) time to emerge from the start box; and 5) time to emerge from the start arm. An arm was recorded as chosen once the end of the lionfish caudal fin fully passed through the maze arm. While lionfish could visit other arms besides the goal arm, a trial was only scored as correct if the goal arm was the first arm selected (Fig. 1). We inferred that learning had occurred if lionfish progressively located the shrimp in the goal arm more quickly over time and if they made fewer errors in later sessions- that is, the number of correct trials increased over time. Similar criteria for learning have been used by other researchers (e.g. Bezzina et al. 2014). Latency to emerge from the start box or 'shelter' was interpreted as a measure of boldness (sensu Sneddon 2003; Cote et al. 2010). Latency to leave the start arm was interpreted as proclivity to explore, that is, their willingness to move in a novel environment (per Cote et al. 2010). For measures of latency, an animal was considered to have left the start arm or start box once half its body moved outside of the arm and/or box. Animals that refused to swim were assigned a latency of 300 s (the total trial time). Shorter latency times indicated higher boldness and exploration. We then tested how the above variables were correlated with learning performance of lionfish.

Maze apparatus

We assessed learning ability by training lionfish to complete a food-reward task using a four-arm plexiglass maze, similar to those used in previous spatial navigation experiments (e.g. Rodriguez et al. 1994;

White and Brown 2014) and to test learning in invasive species (e.g. Chung et al. 2017). Two opaque guillotine doors (50 cm × 32 cm) were used to delineate the start box (positioned 33 cm from the arm end) and to block off the opposite arm, producing a T-maze. Three of the maze arms including the start arm were unrewarded. Doors were attached to a nylon filament and controlled by a pulley system used to raise and lower the doors as needed. Four identical artificial plants (15.5 cm tall; two in each of the possible goal arms) were placed 33 cm from arm ends. Four food holders made of white 1.5-inch PVC were installed in each of the four-maze arm ends and raised 25 cm off the bottom of the tank floor, such that the shrimp were introduced at eye level of the fish. Food holders were installed in all arms to preclude unintentional directional cues. Four sets of extramaze cues were positioned outside the maze but inside the tank arena (Supplementary file). Selected cues were dissimilar in size, shape, colour, and height: (1) oyster shells; (2) small rocks; (3) large rectangular cinder blocks; and (4) stacked assorted cylindrical PVC pipe. These cues served to guide spatial navigation of lionfish through the maze to the food reward.

Navy-coloured electrical tape was placed 9 cm from the bottom of the maze arm along the interior of all arms and delineated maze boundaries. The maze was placed inside a circular tank of 2.13 m diameter and 0.72 m height. The tank interior was covered by a grey plastic tarp to provide contrast for video recording. The maze was filled with water to a height of 30 cm. The arm ends were aerated via single air stones. The entire arena was surrounded by a black plastic tarp to eliminate any global extramaze cues other than those positioned directly outside of the maze itself. Six equidistant carabineers affixed the tarp to a black ceiling. No directional cues could be gleaned from the ceiling.

Trials were recorded via a Go-Pro Hero 5 camera (GoPro Inc., San Mateo, CA, USA) recording at high resolution (1080p, 30 frames per second). The arena was illuminated by a single LED light (Husky: Model K40066). Both the GoPro and light were placed on a square platform suspended from a ceiling-mounted metal rod that was situated in the middle of the experimental arena, thus providing no relevant directional cues to fish. The platform was rotated on several occasions to further reduce this possibility. The Go-Pro was set up to allow trials to be viewed remotely via a paired mobile device.

An acclimatization phase was used to familiarize lionfish with the maze. All maze arms were open during this period and no extramaze cues were present, consistent with previous experiments (e.g. Odling-Smee et al. 2008). Individuals unwilling to swim were left in the maze and allowed to explore for up to 3 h. On average, lionfish spent between 20 and 40 min in the maze. No food was offered during this period. This phase was complete when all fish freely explored the maze. Lionfish completed this phase within three trials.

Once fish freely explored the maze, pre-training trials were conducted to establish an association between the food dispensers, the end of the maze arm, and the shrimp received. Fish were placed in the start box for 2 min before the door was raised after which fish could swim freely through the maze. The arm opposite the starting arm was blocked off via a guillotine door. The start box location was alternated between the start arm and the blocked off arm on every other trial (Fig. 1). Fish were rewarded with shrimp at the end of both goal arms to spur an association between the arm ends and receiving food. Trials in which fish refused to swim were terminated after 1 h. This phase was deemed complete and training trials only commenced once fish made directed movements down one of the goal arms. Fish required a similar number of pre-training trials (mean ± SE = 9.81 ± 0.31). During pre-training trials, we identified place and/or turn preferences. For fish in the simple group, those that displayed a turn preference (e.g. fish that consistently turned right irrespective of whether they started from S1 or S2) were trained in the opposite condition (e.g. trained to turn left). For fish in the complex group, if fish displayed a place preference (that is, they preferred either G1 or G2), they were assigned to the sub-group whose goal location was in the opposite arm. If lionfish exhibited neither turn nor place preferences, they were randomly assigned to sub-groups of their respective spatial navigation strategy. These measures further reduced the likelihood that lionfish oriented using inherent directional biases.

Once all fish completed pre-training trials, training trials commenced. To reach criterion, fish had to make ≥ 70% cumulative correct choices over three consecutive sessions. The likelihood that this criterion

could be obtained by chance is less than 0.05% (χ^2 test, $P < 0.05$). We measured learning speed as the trial number in which fish obtained criterion. During the training phase, one session comprised of five consecutive trials was conducted once every several days. Fish were tested a maximum of twice in any 7-day period. The order in which fish were tested each day was randomized to eliminate order effects; trials for both fish in the complex and simple groups were interspersed on a given day. Food was only provided at the goal arm during training trials. For lionfish in the complex group, the goal position (G1 or G2) remained constant but the starting box alternated between start arm one (S1) and start arm two (S2) (Fig. 1). Fish in this group were not allowed to leave from same starting box more than twice in a row, but whether they were released from S1 or S2 was chosen at random. In the complex strategy group, lionfish were unable to orient using body-centric cues, given that the direction in which lionfish were required to turn changed depending on whether lionfish started from S1 or S2. For lionfish in the simple strategy group, both the goal and start position remained constant (Fig. 1). Fish generally completed all five trials within a session. There were several instances within a session whereby fish refused to leave the start box and complete the task. Across fish, this occurred on average in 3 out of 15 trials. In these cases, and in the absence of any choice, we scored the number of correct trials out of the total number of trials in which fish engaged in the task. Similar criteria for task engagement have been utilized in previous food reward experiments (e.g. Mamuneas et al. 2014).

To initiate a trial, fish were netted from their home tanks, transferred to the start box of the maze and left for 2 min to acclimate. After 2 min, the guillotine door was raised, and the fish could swim freely until it correctly moved through the maze and ate the shrimp, or, if the fish was not motivated to swim, until 5 min elapsed. Shrimp were only introduced to the maze via the food holder when a lionfish reached the end of the goal arm. This ensured that lionfish were not using olfactory, visual, and lateral line prey cues to orient. At the end of a trial, a net was used to guide fish back to the start box where they underwent a 2-min inter-trial interval. Fish were not removed from the maze in between trials. To reduce the likelihood of a fish simply tracing their way from the start box back to the goal arm, fish were forced into other arms on intermittent trials before being guided back to the start box. Many scorpaenids rely on their lateral line as opposed to olfactory cues to detect prey (Montgomery and Hamilton 1997; Bassett et al. 2007). However, we took several precautionary measures to mitigate the influence of olfaction or other cues that may have influenced lionfish. Between trials, water within the maze was mixed. Between sessions, we scrubbed the tank floor and placed air stones within the maze. 10% water changes were also conducted twice weekly.

Fish were tested in training trials until they achieved criterion (as described above) or, if they failed to do so, for a maximum of 15 sessions. Fish that reached criterion were inferred to have learned the food-reward task. Animals that did not reach criterion did not undergo memory retention trials and were considered non-learners. Four fish—two from each of the simple and complex groups—were removed from the experiment owing to an extended unwillingness to leave the start box or move through the maze. Data for these fish were excluded from all analyses. Of the lionfish that reached criterion, we tested memory retention across each of the complex and simple spatial navigation groups in which they were trained. For this phase, individuals were randomly selected to undergo training breaks from five up to a maximum of 42 days. They were then assigned to one of three training break periods: short (up to 7 days), medium (from 8 to 14 days), or long (more than 2 weeks). Of the fish that reached criterion, each was subject to at least one training break. During a training break, lionfish were kept in their housing tanks with conspecifics. At the end of each training break, fish were reintroduced to the maze and their performance was evaluated over a single session (i.e. their ability to successfully complete the task and locate the food reward). We compared the performance of each fish during the memory retention test with that from the session that immediately preceded the training break. If fish displayed remedial performances, additional sessions were conducted to determine the time required to regain the level of performance that preceded the training break.

Statistical analyses

Invasive species often exhibit correlated suites of behaviours (i.e. behavioural syndromes, Sih and Del Giudice 2012). We used Spearman's rank correlation

to test for correlations among the behavioural traits (speed, boldness, and exploratory behaviour). Positive correlations between the variables indicate a behavioural syndrome. We also analysed the consistency of these variables over time—across sessions—using Spearman's rank correlation, for both fish that learned the task and those that did not.

We used generalized linear mixed models (GLMMs), linear mixed models (LMMs), and basic statistics to explore patterns of learning and behaviour over time across experimental groups (simple and complex), and between individual fish [packages *lme4* (Bates et al. 2014); *stats* (R Core Team 2018)]. Animal ID was included as a random effect across all models to account for repeated use of the same fish across trials. We assessed model parsimony via Akaike's Information Criterion (AIC). Tukey-adjusted multiple comparisons were conducted post hoc. Normality of residuals for LMMs was confirmed visually by inspection of Q–Q plots. Owing to non-normality of the response variable *time to task completion*, it was rank transformed before being analyzed with a one-way repeated measures ANOVA.

Data are presented separately for all fish that completed the experiment (N = 16) and for those that learned the food-reward task (N = 8). As there were no significant differences in performance between the counter-balanced conditions of each of the complex and simple navigation groups (*complex*; Wilcoxon rank-sum test: W = 1775.5, $P > 0.10$, *simple*; Wilcoxon rank-sum test: W = 2141.5, $P > 0.10$), we collapsed the individual sub-groups and report results for each group inclusive of their respective sub-groups.

To assess memory retention, we conducted comparisons between fish performance after the training break with that directly before it using paired t tests. A Shapiro–Wilk normality test confirmed that the differences of the pairs followed a normal distribution. If the performance of a lionfish during the memory retention test was significantly lower than that directly preceding the training break, we considered the training break to have exceeded their maximum memory duration. Fish that retained memory of the shrimp's location should have performed equal to or better than before the training break. All statistical analyses were conducted in R, version 3.5.2 (R Core Team 2018).

Results

The three behavioural traits tested were significantly positively correlated to each other (Table 1). All behavioural traits tested also showed significant repeatability across trials, highlighting their consistency over time, for both learners and non-learners, indicating a behavioural syndrome (Table 2).

Lionfish that demonstrated superior learning abilities and reached criterion completed the task more quickly, more accurately, were bolder, and more exploratory than those that did not (Table 3). However, performance between learners and non-learners was unrelated to the spatial navigation strategy in which they were trained (criterion*group interaction LMM: estimate ± SE = 3.04 ± 13.54, $P > 0.10$). When controlling for the effect of spatial navigation group, lionfish that learned the task made significantly more correct choices relative to those that did not over consecutive sessions (Fig. 2, Table 4). Bolder fish had better performances relative to their shyer counterparts. However, neither speed (time until task completion) nor exploratory tendency predicted performance.

Time until task completion decreased significantly across trials (Table 5). Over time, lionfish that learned the task took significantly less time to navigate the maze relative and those that failed to do so. Fish that were bolder had shorter trial durations than their shyer counterparts, though exploratory tendency had no effect on time until task completion. Trial duration also did not differ between complex and simple spatial navigation groups either over time or on average (Wilcoxon: W = 35,076, $P > 0.10$). Over time, lionfish chose the goal arm significantly more often relative to alternative arms, whose choice was not predicted by exploratory tendency or spatial navigation group (Table 5). Bolder lionfish and those that reached criterion chose the goal arm first significantly more than shyer fish and poorer learners, respectively.

Eight of 16 fish that participated in the full course of the experiment reached criterion (N = 3 complex strategy; N = 5 simple strategy). There was no significant difference between fishes in the number of sessions required to reach criterion (mean ± SE = 10.63 ± 1.39; Kruskal–Wallis test: df = 7, $P > 0.10$), nor in performance between spatial navigation groups (Table 3).

Table 1 Spearman's rank correlations between the three behaviours measured during the learning assay

	Boldness	Exploratory behaviour
Exploratory behaviour	0.65, $P < 0.0001$*	
Speed	0.44, $P < 0.0001$*	0.34, $P = 0.0001$*

Asterisks indicate significant values ($P \leq 0.05$)

Table 2 Behavioural consistency of the three behaviours measured in the learning assay, taken across trials, as a function of both fish that learned the task and failed to do so

	Rank consistency (learners)	Rank consistency (non-learners)
Exploratory behaviour	− 0.35, $P < 0.0001$*	− 0.41, $P < 0.0001$*
Speed	− 0.71, $P < 0.0001$*	− 0.47, $P < 0.0001$*
Boldness	− 0.34, $P < 0.0001$*	− 0.43, $P < 0.0001$*

Asterisks indicate significant values ($P \leq 0.05$)

Table 3 Behavioural differences among lionfish compared via a two-sample t test

	Learners versus non-learners	Simple versus complex Spatial navigation group
Time to task completion	t = 4.80, df = 229, $P < 0.0001$*	t = − 0.89, df = 133, $P > 0.10$
Performance	t = − 5.82, df = 241, $P < 0.0001$*	t = − 1.16, df = 133, $P > 0.10$
Boldness	t = 1.61, df = 220, $P = 0.05$*	t = − 1.37, df = 122, $P > 0.10$
Exploratory behaviour	t = 2.16, df = 220, $P < 0.05$*	t = − 0.94, df = 122, $P > 0.10$

Separate columns display (1) comparisons between fish that learned to complete the food-reward task and those that failed to do so; (2) comparisons between simple and complex spatial navigation groups of lionfish that reached criterion

Asterisks indicate significant values ($P \leq 0.05$)

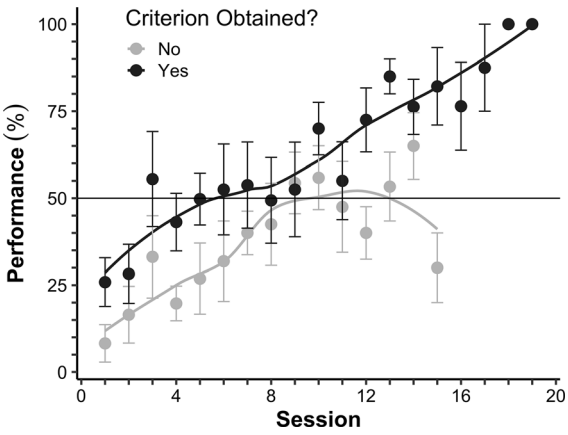

Fig. 2 Mean (± SE) performance (percent correct choices) of lionfish (N = 16) across repeated sessions. Data are inclusive of all lionfish that participated in the study, separated by fish that learned and failed to learn the food-reward task

Spatial navigation groups were similar with respect to time until task completion, exploratory tendency, and boldness (Table 3). However, performance in the simple navigation group improved more quickly and was less variable over time relative to those in the complex group (session * spatial navigation group interaction LMM: 1.95 ± 0.93, $P < 0.05$) (Fig. 3). There were also significant interactive effects between spatial navigation group and both speed and exploratory behaviour (Table 6). For lionfish that were highly exploratory and fast, those in the complex group had poorer performances relative to fish in the simple navigation group. Conversely, performance was similar between spatial navigation groups for slow neophobic fish.

All fish (N = 8) that reached criterion were subject to training breaks to assess their memory duration.

Table 4 Behavioural and time effects on lionfish performance (percent correct choices) from a linear mixed model

	Statistical test
Session number	2.94 ± 0.56, $P < 0.0001$*
Time to task completion	− 0.02 ± 0.03, $P > 0.10$
Criterion status (learned/failed to learn)	14.80 ± 6.58, $P < 0.05$*
Boldness	− 0.08 ± 0.04, $P = 0.06$
Exploratory behaviour	0.01 ± 0.03, $P > 0.10$

Results are plotted as estimates ± SE

Asterisks indicate significant values ($P \leq 0.05$)

Table 5 Behaviour, group, and time effects on (1) time to task completion using a repeated measures ANOVA; (2) first arm chosen (goal arm/other arm) using a GLMM with binomial error structure

	Time to task completion	First arm chosen
Trial number	$F_{1,990} = 253.7$, $P < 0.0001$*	0.02 ± 0.003, $P < 0.0001$*
Spatial navigation group	$F_{1,13} = 0.1$, $P > 0.10$	0.23 ± 0.24, $P > 0.10$
Criterion status	$F_{1,13} = 10.0$, $P = 0.01$*	0.56 ± 0.24, $P < 0.05$*
Boldness	$F_{1,990} = 55.2$, $P < 0.0001$*	− 0.25 ± 0.09, $P = 0.01$*
Exploratory behaviour	$F_{1,990} = 1.1$, $P > 0.10$	0.05 ± 0.08, $P > 0.10$

Results of the GLMM are denoted as estimates ± SE

Asterisks indicate significant values ($P \leq 0.05$)

Irrespective of the length of training break and spatial navigation group, lionfish remembered and were able to successfully navigate to the goal arm to a maximum of 6 weeks—the maximum tested period—in the absence of reinforcement. Across fish, performance during the memory retention test was better than that directly preceding training breaks (paired t test: $t_{18} = 2.19$, $P < 0.05$), suggesting memory consolidation.

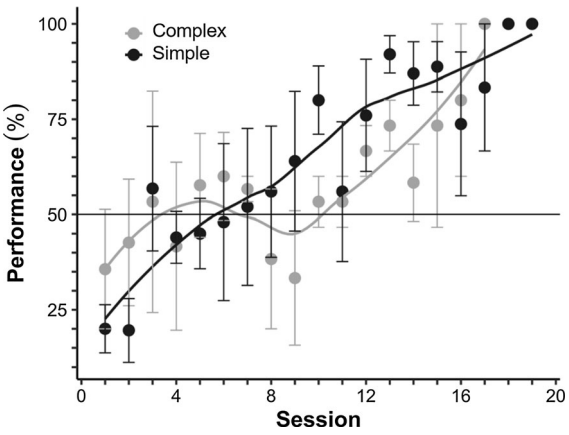

Fig. 3 Mean (± SE) performance (percent correct choices) by lionfish as a function of repeated sessions, separated by spatial navigation group. Only those fish that reached criterion are plotted

Discussion

In our study, the presence of behavioural traits influenced an ecologically significant behaviour—learning the location of an optimal foraging site. Our results suggest that individual differences in lionfish learning ability may be in part explained by variability in behavioural traits, which were both correlated with one another and consistent over time (Tables 1, 2).

Superior learners were faster, bolder, and more exploratory than their counterparts and more readily exploited foraging sites with prey (Table 4). The aforementioned traits appear to be regular predictors of spatial learning and have been positively correlated with performance in prior experiments (e.g. Dugatkin and Alfieri 2003; Boogert et al. 2006; Carere and Locurto 2011). Boldness and neophilia have similarly

Table 6 Interactive behaviour and group effects on lionfish performance (percent choices correct) from a linear mixed model

Results are plotted as estimates ± SE

Asterisks indicate significant values ($P \leq 0.05$)

	All fish	Learners
Group * exploratory behaviour	− 20.7 ± 9.4, $P < 0.05$*	− 24.2 ± 12.4, $P = 0.07$
Exploratory fish		
Group * exploratory behaviour	18.4 ± 14.3, $P > 0.10$	29.9 ± 20.4, $P > 0.10$
Neophobic fish		
Group * time to task completion	− 23.8 ± 9.7, $P < 0.05$*	− 23.8 ± 9.7, $P < 0.05$*
Fast fish		
Group * time to task completion	7.99 ± 10.0, $P > 0.10$	15.2 ± 14.4, $P > 0.10$

been associated with competitive superiority and invasion potential in other non-indigenous species (Holway and Suarez 1999; Pintor et al. 2009).

We observed substantial trait variation among lionfish, which may be indicative of ecologically relevant differences between individuals. Akin to other species, lab-derived behavioural variation shown here may reflect differences in foraging behaviour in the wild (Herborn et al. 2010). This admixture of phenotypes may subsequently promote greater group foraging efficiency relative to those comprised of monotypes (Dyer et al. 2009). With respect to invasive species, within-species polymorphism in phenotype may similarly affect invasion dynamics (e.g. Pintor et al. 2009; Cote et al. 2010), as it is likely to influence an invasive species' rate of spread and subsequently impact (Duckworth and Badyaev 2007; Cote et al. 2010; Fogarty et al. 2011).

Invasive species' context-dependent ecological impacts have garnered substantial interest, the inherent variability of which can impede accurate prediction and understanding (Ricciardi et al. 2013). Accordingly, lionfish impacts throughout their invaded range have been somewhat variable (Côté and Smith 2018). Understanding the mechanisms underpinning this variability is of utmost importance to guide effective management (Ingeman et al. 2017). Thus, the relative composition of behaviours within a population may contribute to this species' context-dependent impacts and heterogeneity in observed impacts throughout their invaded range. The presence of this phenomenon demands further study.

Population persistence in ephemeral environments is contingent on dispersing to and exploiting patchily distributed resources. While dispersal tendency per se was not measured in our study, the suite of traits we investigated co-vary with dispersal tendency in other invasive species (e.g. Rehage and Sih 2004; Cote et al. 2010). Ultimately, understanding inter-individual variation in behaviour may help researchers better understand fish movement dynamics (Spiegel et al. 2017), which is particularly important in invasive species management and prevention. These traits could therefore serve to better understand invasion dynamics of lionfish throughout their invaded range. Behavioural assays can likewise serve as a way to assess the potential impact and dispersal tendency of new invaders.

We observed no difference between spatial navigation groups with respect to learning rate—the number of sessions required to reach criterion—consistent with previous findings (e.g. Rodriguez et al. 1994). We also found no effect of spatial navigation group on performance over time, suggesting that lionfish may be able to navigate using multiple strategies. However, while faster and more exploratory lionfish were better learners, performance of fish using the complex strategy was more variable relative to those trained in the simple strategy. Conversely, slower or neophobic fish did not differ in their performance between navigation groups (Table 6). This implies that fast exploratory fish may have assimilated limited information about their environment; thus, when their spatial location relative to the food changed, they were unable to adapt. These findings are consistent with Sih and Del Giudice's (2012) speed-accuracy trade-off.

All lionfish retained memories of the food reward location for up to 6 weeks in the absence of reinforcement. These memory spans are analogous to those demonstrated in other species (e.g. Schluessel and Bleckmann 2012). However, memory duration in fishes is still poorly understood (Ingraham et al. 2016), providing a ripe avenue for future research. Remembering food locations in nature is important (Hughes et al. 1992; Odling-Smee et al. 2008), as superior

memory retention may predispose animals to better quality and greater quantities of food (Odling-Smee and Braithwaite 2003). This is particularly important for invasive species, whose superior memory spans have been reported relative to native species (reviewed in Weis 2010). Whether superior learning and memory can serve as a means by which lionfish outcompete co-occurring natives is contingent on knowledge of learning speed and memory retention in native predators, information we currently lack.

We are cautious to conclusively summarize lionfish learning and memory ability considering the uncertainties inherent with small sample sizes, though our sizes were analogous to those used in previous experiments (Brown and Braithwaite 2004; Schluessel and Bleckmann 2012). Furthermore, given the absence of probe trials we cannot exclude the possibility that lionfish oriented through other mechanisms not proposed here. Nonetheless, our results appear to indicate that lionfish are capable of spatial learning irrespective of the strategy in which they are trained, whose abilities may be mediated by behavioural traits.

The presence of conspecifics may bolster an animal's learning ability via social learning (McAroe et al. 2017). Social learning is readily used by fish (Brown and Laland 2003) and may mediate invasive species' success (Damas-Moreira et al. 2018). Lionfish appear to exploit information about patch quality from conspecifics (DeRoy et al. 2020), which may permit increased foraging efficiency and exploitation of available resources in nature. Indeed, learning-based foraging efficiency is thought to influence the success of invasive species (Weis 2010). However, the degree to which learning in lionfish is affected by the presence of conspecifics—and how this affects their impact—is presently unknown. Future research should endeavour to understand the relationship between social learning and behaviour in this and other invasive species, and its implications for ecological impact.

Conclusion

This study adds to the burgeoning body of literature investigating learning and memory in invasive species and provides a framework on which subsequent research can build. Understanding invasive species' behaviour may contribute to a more meaningful understanding of their impact. More broadly, behaviourally-mediated learning and foraging efficiency may help to unravel the mechanisms underlying invasiveness. Our study suggests that food-related memories in this invasive species are robust, and indicates a link between their temperament and basic learning ability. Future research should assess the presence of behavioural phenotypes in this species, and their relation to dispersal and ecological impact in field populations.

Acknowledgements We thank Florida Gulf Coast University and Vester Marine and Environmental Science Research Field Station for use of facilities and assistance provided throughout the study. We also thank the Vester Field Station facilities' manager Bob Wasno for his help during the study. All fish were handled and collected under a Letter of Acknowledgement (LOA) from the National Oceanic and Atmospheric Administration (NOAA) and were handled with approval of University of Windsor's Animal Care Committee (AUPP #17-01) and in accordance with the Canadian Council on Animal Care. This work was supported by a Natural Sciences and Engineering Research Council of Canada Michael Smith Foreign Study Supplement, Natural Sciences and Engineering Research Council of Canada Canada Graduate Scholarship-Master's; a Mitacs Globalink Research Award to E.M.D; Natural Sciences and Engineering Research Council of Canada Discovery Grants to N.E.H. and H.J.M; and a Canada Research Chair in Aquatic Invasive Species to H.J.M.

References

Albins MA (2013) Effects of invasive Pacific red lionfish *Pterois volitans* versus a native predator on Bahamian coral-reef fish communities. Biol Invasions 15:29–43. https://doi.org/10.1007/s10530-012-0266-1

Bassett DK, Carton AG, Montgomery JC (2007) Saltatory search in a lateral line predator. J Fish Biol 70:1148–1160. https://doi.org/10.1111/j.1095-8649.2007.01380.x

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4: R package version 1.1–7 [Computer software]. Accessed from https://cran.r-project.org/web/packages/lme4/index.html

Bell WJ (1991) Searching behaviour: the behavioural ecology of finding resources. Chapman and Hall, London

Bezzina CN, Amiel JJ, Shine R (2014) Does invasion success reflect superior cognitive ability? A case study of two congeneric lizard species (*Lampropholis, Scincidae*). PLoS ONE 9:e86271. https://doi.org/10.1371/journal.pone.0086271

Boogert NJ, Reader SM, Laland KN (2006) The relation between social rank, neophobia and individual learning in starlings. Anim Behav 72:1229–1239. https://doi.org/10.1016/j.anbehav.2006.02.021

Brown C (2012) Experience and learning in changing environments. In: Candolin U, Wong BBM (eds) Behavioural

responses to a changing world. Oxford University Press, Oxford UK, pp 46–60
Brown C, Braithwaite VA (2004) Effects of predation pressure on the cognitive ability of the poeciliid *Brachyraphis episcopi*. Behav Ecol 16:482–487. https://doi.org/10.1093/beheco/ari016
Brown C, Laland KN (2003) Social learning in fishes: a review. Fish Fish 4:280–288. https://doi.org/10.1046/j.1467-2979.2003.00122.x
Brydges NM, Colegrave N, Heathcote RJ, Braithwaite VA (2008) Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. J Anim Ecol 77:229–235. https://doi.org/10.1111/j.1365-2656.2007.01343.x
Carere C, Locurto C (2011) Interaction between animal personality and animal cognition. Curr Zool 57:491–498. https://doi.org/10.1093/czoolo/57.4.491
Chapple DG, Simmonds SM, Wong BB (2012) Can behavioural and personality traits influence the success of unintentional species introductions? Trends Ecol Evol 27:57–64. https://doi.org/10.1016/j.tree.2011.09.010
Chung M, Goulet CT, Michelangeli M, Melki-Wegner B, Wong BB, Chapple DG (2017) Does personality influence learning? A case study in an invasive lizard. Oecologia 185:641–651. https://doi.org/10.1007/s00442-017-3975-4
Côté IM, Smith NS (2018) The lionfish Pterois sp. invasion: has the worst-case scenario come to pass? J Fish Biol 92:660–689. https://doi.org/10.1111/jfb.13544
Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). Proc R Soc B Biol Sci 277:1571–1579. https://doi.org/10.1098/rspb.2009.2128
Damas-Moreira I, Oliveira D, Santos JL, Riley JL, Harris DJ, Whiting MJ (2018) Learning from others: an invasive lizard uses social information from both conspecifics and heterospecifics. Biol Lett 14:20180532. https://doi.org/10.1098/rsbl.2018.0532
DeRoy EM, Scott R, Hussey NE, MacIsaac HJ (2020) Density dependence mediates the ecological impact of an invasive fish. Divers Distrib 00:1–14. https://doi.org/10.1111/ddi.13063
Duckworth RA, Badyaev AV (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. Proc Natl Acad Sci 104:15017–15022. https://doi.org/10.1073/pnas.0706174104
Dugatkin LA, Alfieri MS (2003) Boldness, behavioural inhibition and learning. Ethol Ecol Evol 15:43–49. https://doi.org/10.1080/08927014.2003.9522689
Dukas R (2004) Evolutionary biology of animal cognition. Annu Rev Ecol Evol Syst 35:347–374. https://doi.org/10.1146/annurev.ecolsys.35.112202.130152
Dyer JRG, Croft DP, Morrell LJ, Krause J (2009) Shoal composition determines foraging success in the guppy. Behav Ecol 20:165–171. https://doi.org/10.1093/beheco/arn129
Fogarty S, Cote J, Sih A (2011) Social personality polymorphism and the spread of invasive species: a model. Am Nat 177:273–287. https://doi.org/10.1086/658174
Herborn KA, Macleod R, Miles WT, Schofield AN, Alexander L, Arnold KE (2010) Personality in captivity reflects personality in the wild. Anim Behav 79:835–843. https://doi.org/10.1016/j.anbehav.2009.12.026
Holway DA, Suarez AV (1999) Animal behaviour: an essential component of invasion biology. Trends Ecol Evol 14:328–330. https://doi.org/10.1016/S0169-5347(99)01636-5
Hughes RN, Kaiser MJ, Mackney PA, Warburton K (1992) Optimizing foraging behaviour through learning. J Fish Biol 41:77–91. https://doi.org/10.1111/j.1095-8649.1992.tb03870.x
Ingeman KE, Albins MA, Benkwitt CE, Green SJ, Kindinger TL, Tuttle LJ, Hixon MA (2017) Resolving differences in observed impacts of invasive lionfish and clarifying advice to managers. PeerJ Preprints 5:e3455v1. https://doi.org/10.7287/peerj.preprints.3455v1
Ingraham E, Anderson ND, Hurd PL, Hamilton TJ (2016) Twelve-day reinforcement-based memory retention in African cichlids (*Labidochromis caeruleus*). Front Behav Neurosci 10:157. https://doi.org/10.3389/fnbeh.2016.00157
Mamuneas D, Spence AJ, Manica A, King AJ (2014) Bolder stickleback fish make faster decisions, but they are not less accurate. Behav Ecol 26:91–96. https://doi.org/10.1093/beheco/aru160
McAroe CL, Craig CM, Holland RA (2017) Shoaling promotes place over response learning but does not facilitate individual learning of that strategy in zebrafish (*Danio rerio*). BMC Zool 2:10. https://doi.org/10.1186/s40850-017-0019-9
Montgomery JC, Hamilton AR (1997) Sensory contributions to nocturnal prey capture in the dwarf scorpion fish (*Scorpaena papillosus*). Mar Freshw Behav Phy 30:209–223. https://doi.org/10.1080/10236249709379026
Odling-Smee LC, Braithwaite VA (2003) The role of learning in fish orientation. Fish Fish 4:235–246. https://doi.org/10.1046/j.1467-2979.2003.00127.x
Odling-Smee LC, Boughman JW, Braithwaite VA (2008) Sympatric species of threespine stickleback differ in their performance in a spatial learning task. Behav Ecol Sociobiol 62:1935–1945. https://doi.org/10.1007/s00265-008-0625-1
O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Oxford University Press, Oxford
Pintor LM, Sih A, Kerby JL (2009) Behavioural correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology 90:581–587. https://doi.org/10.1890/08-0552.1
Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and health. Annu Rev Env Resour 35:25–55. https://doi.org/10.1146/annurev-environ-033009-095548
R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.R-project.org/
Raine NE, Chittka L (2008) The correlation of learning speed and natural foraging success in bumble-bees. Proc R Soc B 275:803–808. https://doi.org/10.1098/rspb.2007.1652
Rehage JS, Sih A (2004) Dispersal behavior, boldness, and the link to invasiveness: a comparison of four Gambusia species. Biol Invasions 6:379–391. https://doi.org/10.1023/B:BINV.0000034618.93140.a5
Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of

nonnative species. Ecol Monogr 83:263–282. https://doi.org/10.1890/13-0183.1

Rodriguez F, Duran E, Vargas JP, Torres B, Salas C (1994) Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. Anim Learn Behav 22:409–420. https://doi.org/10.3758/BF03209160

Schluessel V, Bleckmann H (2012) Spatial learning and memory retention in the grey bamboo shark (*Chiloscyllium griseum*). Zoology 115:346–353. https://doi.org/10.1016/j.zool.2012.05.001

Schoener TW (1983) Field experiments on interspecific competition. Am Nat 122:240–285. https://doi.org/10.1086/284133

Schofield PJ (2010) Update of geographic spread of lionfishes [*Pterois volitans* (Linnaeus, 1758) and *P. miles* (Bennett, 1828)] in the western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquat Invasions 5(Supplement 1):S117–S122. www.aquaticinvasions.net/2010/Supplement/AI_2010_5_S1_Schofield.pdf

Sih A, Del Giudice M (2012) Linking behavioural syndromes and cognition: a behavioural ecology perspective. Philos Trans R Soc B 367:2762–2772. https://doi.org/10.1098/rstb.2012.0216

Sneddon LU (2003) The bold and the shy: individual differences in rainbow trout. J Fish Biol 62:971–975. https://doi.org/10.1046/j.1095-8649.2003.00084.x

Spiegel O, Leu ST, Bull CM, Sih A (2017) What's your move? Movement as a link between personality and spatial dynamics in animal populations. Ecol Lett 20:3–18. https://doi.org/10.1111/ele.12708

Van Gerven DJH et al (2012) Direct measurement of spontaneous strategy selection in a virtual Morris water maze shows females choose an allocentric strategy at least as often as males do. Behav Neurosci 126:465–478. https://doi.org/10.1037/a0027992

Warburton K (2003) Learning of foraging skills by fish. Fish Fish 4:203–216. https://doi.org/10.1046/j.1467-2979.2003.00125.x

Weis J (2010) The role of behaviour in the success of invasive crustaceans. Mar Freshw Behav Phy 43:83–98. https://doi.org/10.1080/10236244.2010.480838

White GE, Brown C (2014) A comparison of spatial learning and memory capabilities in intertidal gobies. Behav Ecol Sociobiol 68:1393–1401. https://doi.org/10.1007/s00265-014-1747-2

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.