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Title: Linking phenotypic correlations from a diverse set of laboratory tests to field behaviours in the crayfish, Orconectes virilis.

Running Title: Laboratory correlates to field behavior

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Abstract: The presence of phenotypic behavioral correlations and their connection to fitness consequences of organisms has received considerable debate within the literature. Yet, little work has been done to connect any behavioural correlates found within a set of laboratory studies to natural behaviour observed under complex environmental conditions. To help fill this gap, individual crayfish, collected from the same local population, completed five different behavioural assays in a laboratory setting in a random order. These data were used to reveal any possible correlations for behavioral scores across all of the laboratory tests. Subsequently, these same individuals were placed into the field and video recorded for 24 hours. A separate set of field behaviours, related to the laboratory assays, were quantified from the field videos. The normalized laboratory and field behaviours were used in three step-wise statistical analyses. First, normalized data were loaded into a PCA to generate *a priori* hypotheses on potential behavioural correlates. These hypotheses were subsequently tested using general multiple linear regression. Finally, structural equation modeling (SEM) was performed to elucidate any behavioral modules from the laboratory assays that correlated with behavioural patterns present from the fieldwork. Three laboratory-based behavioural modules were connected to three separate field assays: exploration-avoidance, bold-shy, and aggressiveness. Yet, some behaviors exhibited in the laboratory assays were uncorrelated with any behaviors found in the field and vice versa. Results from this study provide evidence that although many different behavioural correlates may exist within laboratory settings, these same modules may not translate directly into predicting behaviour under natural settings.

Keywords: structural equation modeling; behavioural phenotypes; crayfish; field behavior

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Animals exhibit a wide suite of behaviours that vary depending on the context, but are also correlated between contexts (Gosling, 2001; Réale et al., 2000; Sih and Chadwick, 2004 a, b; Wilson et al., 1994). Within a population, individuals often exhibit a range of behaviours along a continuum. For example, on a boldness continuum, certain individuals will be consistently bolder or shyer than others. However, the behaviour exhibited by individuals varies across contexts, creating an individual derived continuum of behaviour nested within the overall consistent population distribution along a behavioural continuum (Dall et al., 2004; Sih and Chadwick, 2004a). These individually plastic behaviours can be termed contextual plasticities because the exact behavioural phenotypic display is dependent upon the context (Stamps, 2015). Domesticated rainbow trout take more risks when foraging relative to native fish that are subject to higher predation within their habitats (Wilson and Stevens, 2005). This increased level of risk demonstrated by the domesticated fish led to increased foraging and body size, which was beneficial for those organisms from a fitness standpoint. Conversely, when placed in habitats with similar predation risks, domesticated fish continued to take more risks than the natives, increasing their level of mortality (Biro et al., 2004). Even though changing the context (e.g. habitat with low or high predation risk) may allow for a shift in risk taking behaviour or "riskiness" for some individuals, these same animals are still "riskier" individuals than the average population response. In those situations where a single animal displays a consistent pattern of behaviour across different tasks (i.e. stays within a certain range on the population continuum), the pattern of consistency within the individual animal has been termed a behavioural syndrome (Sih and Chadwick, 2004 a, b).

Although contextual plasticity is usually measured along a variation in a single variable, the contextual complexity of natural environments would dictate a multi-dimensional plasticity (Westneat et al., 2011, Dosmann and Mateo, 2014; Westneat et al., 2015). In the guppy (*Poecilia reticulate*), females prefer males who exhibit behaviours denoting bold individuals such as those willing to approach predators (Godin and Dugatkin, 1996). Mosquito fish that are on the higher end of the sociability continuum (i.e. are more social) disperse farther distances than less social fish, allowing them to access resources that are not available to less social fish (Cote et al., 2010). More active males (i.e., more time spent swimming and less time freezing in open spaces) provided more brood care in convict cichlids (Budaev et al., 1999). Within complex environments with often competing behavioral interests (attractive and aversive stimuli),

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behavioural plasticity might be displayed differently and have different ecological benefits and costs than what is found under simpler laboratory settings. Certainly, these behavioural correlates have fitness consequences that have been seen in species that range from bighorn sheep, spiders, fish, lizards, and rodents (Dingemanse and Réale, 2005; Smith and Blumstein, 2008; Stamps and Groothius, 2010). Consequently, understanding the different environmental contexts, which may favor certain behavioral phenotypes over others, is an area where additional research is needed.

The distribution of animals within any singular behavioural phenotype has been found to be heavily dependent upon conditions within an ecological environment (Milinski et al., 1997; Wolf and Weissing, 2012). For example, within fish populations, the boldness expressed within a population of animals is influenced by the level of predation risk within the environment (Archard and Braithwaite, 2011; Brydges et al., 2008; Dingemanse et al., 2007; Huntingford, 1976). In addition, differences in predation risk may cause correlations in behavioral patterns that do not exist with populations under less predation risk (Bell and Sih 2007; Dingemanse et al., 2007). Thus, even the presence of specific phenotypes may be context and environment dependent. Thus, a connection between laboratory testing and natural conditions is needed.

A concern of field studies currently being performed is the aspect of changing environmental conditions within the lifespan of the study animal (Stamps and Groothius, 2010). Additionally, Stamps and Groothius (2010) point out that any assumptions about the stability of individual personality differences in animals based on laboratory trials remains untested. Ecosystem level interactions are highly dependent on the behavior of organisms within different niches (Schwartz et al., 2000). The expressions of these behaviours are responses to various situations that should attempt to maximize the overall fitness of an individual. Therefore, an assumption for relevance of behavioural phenotypes in nature is to display appropriate behaviours that increase survival or reproductive success. Given that environmental context influences the expression of personalities, both within and across lifetimes, the quantification and stability of animal phenotypes across environmental context seems to be a critical point (Wolf and Weissing, 2012).

An initial solution to these concerns would be the establishment of behavioral phenotypic structures in controlled laboratory setting that are then examined in conjunction with any potential similar structures seen under real environmental conditions. The purpose of our study was to implement this solution via the examination of behavioural phenotypes within both

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laboratory and field settings to determine whether laboratory assays are connected to field behaviours. To test this linkage between lab and field behaviours, we utilized crayfish (Orconectes virilis) as a model organism. The behavioral repertoire of O. virilis is complex and has been well studied (Bergman et al., 2003; Bergman and Moore, 2003; Cook and Moore, 2008; Gherardi, 2002; Hamr, 2002). Additionally, these animals have been studied in the field under natural conditions in a variety of habitats (Bergman and Moore, 2003; Martin and Moore, 2007). Finally, the nervous system that underlies a number of these behaviors has been extensively studied such that environmental influences on neural activity are well known (Herberholz et al., 2001; Yeh et al., 1996). We hypothesize that laboratory tests requiring similar behaviors (exploration, riskiness, and aggression) will elicit individuals that rank in similar fashion on those tests. Specifically, we expect to find similar population wide rankings (similar individuals scoring high and another set of individuals scoring low) with behavioral tests involving anti-predator responses and exploration behaviors. We also hypothesize that individuals scoring similarly on laboratory tests will score similarly during the field test that involves similar behaviors (exploration and anti-predatory behavior).

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METHODS:

Animals

Twenty – five, reproductive form II female crayfish, *Orconectes virilis*, with completely intact appendages were collected from Maple Bay (Latitude: 45. 4873, Longitude: -84.7065) on Burt Lake (Emmet County, MI, USA). Form II is the non-reproductive form for female crayfish. Crayfish were transported to the Experimental Stream Research Facility at the University of Michigan Biological Station (UMBS) in Pellston, Michigan. Only females were used in this study to eliminate possible sex differences. The carapace and chelae length were measured for all individuals (average carapace length ± SEM: 3.4 ± 0.25 cm; average chelae length ± SEM: 3.1 ± 0.42 cm). Given this size distribution, most of these animals are between two to three years old. Typical lifespans for these animals in this area are three years. Crayfish were housed at the stream laboratory at UMBS in large metal troughs with continuous flow of unfiltered natural river water from the East Branch of the Maple River adjacent to the facility. All individuals were physically, but not chemically, isolated in individual plastic containers (725 ml, 36.6 x 36.6 x 10.1 cm). Animals were kept under ambient temperatures depending on the natural stream water (20˚C to 23˚C) and a natural light/dark cycle during June and August of 2015. All crayfish were marked with white correctional fluid on their carapace used as a non-toxic marker that did not affect behavior to discern individuals during video recordings (Bergman et al., 2003; Bergman and Moore 2004). Crayfish consumed natural detritus within the stream water that fed the housing troughs.

Experimental Protocol

Crayfish were run through five separate laboratory behavioural assays (described below) one per day for five consecutive days. Preliminary tests demonstrated that this structure of testing produces similar results to behavioral tests that allowed recovery time between each test. Thus, to maximize the number of field tests that could be performed during the limited field season, we chose to run behavioral tests on consecutive days. Each animal was only used once in each assay. Once an individual crayfish completed all five laboratory behavioural assays, the crayfish was placed in the final field assay (described below). The order in which each crayfish ran through all the laboratory assays was randomized to prevent any order effect on the results. The individuals went through assays in a random order to also mitigate any motivational differences related to differential timing of the last protein meal. All tanks were filled with fresh

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stream water (same as housing troughs) daily from the Maple River adjacent to the stream lab facility, and each tank was rinsed before another animal went through the assay. A 15-minute acclimation period was used for all assays, unless otherwise stated and all tanks were uniformly lit from above with fluorescent lighting. All behaviours were recorded with either a DVR (SWDVR-43000H) or a camcorder (SONY Handycam HDR-CX405).

Exploration Assays

Arena

To determine the exploration behaviour of individuals, crayfish were placed into an arena that was designed to allow free movement of individuals. The exploration tests were performed in a 76.2 cm x 63.5 cm x 15.24 cm black Plexiglas arena, with black gravel, similar to arenas used in previous crayfish work (Basil and Sandeman, 2000). Black substrate was glued on the bottom of the tank to create a backdrop for a movement-tracking program to better detect the animal's location (Figure 1 A). The arena had four additional walls extending 10.2 cm into the tank at 90° degree angles at the middle of the four walls, 38.1 cm or 31.8 cm from the end of the wall to add dimensionality to the arena.

Protocol

Animals were acclimated under a 14 x 14 cm square cage made from egg crating in the middle of the tank for 15 minutes. After the acclimation period, the cage was lifted and the animal was allowed to explore the arena for a total of 15 minutes. After 15 min, the crayfish was then removed from the tank and the tank was rinsed with stream water to eliminate any lingering chemical cues left that may affect the assay of the next individual.

Behavioural Analysis

Ethovision Noldus XT, a motion tracking software, was used to track a single point on the crayfish's carapace at each second in time. Previous work has shown that a single point per second is an accurate rate for movement and orientation studies (Kamran and Moore, 2015). This generated an x, y coordinate arena location for the crayfish during each second of the exploration assay. An in-house data analysis program was written to calculate each of the behavioural measures for this section. A 35 x 35 cm square grid was digitally superimposed over the exploration data and percentage of the tank exploration was calculated by dividing the total number of grid squares entered by the crayfish by the total number of grid squares (1,225). The

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percentage of the time (s) spent moving was calculated by using a similar method to other orientation studies (Moore et al., Bergner, 2015). We divided the total time the crayfish spent above a minimum movement speed (0.5 cm/s) by the total assay length. Finally, the total time (s) spent near the wall was calculated by determining when the crayfish was within ½ of a body length from any wall. The time a crayfish touching the wall with at least one chelae is a characteristic type of exploration by crustaceans (thigmotaxis) and is different than open exploratory behaviour. A detailed description of all the behaviors across all of the assays is found in table 1.

Conflicting Cues Assay

Arena

To determine the effect of predatory odour on foraging behaviour, crayfish were placed into an arena that was designed to present conflicting cues (food and predatory odour). The tank was 63 x 28 x 14 cm in dimension (Figure 1 B). A PVC pipe cut in half lengthwise, which is used as a shelter, was secured in one corner of the tank. In the center of the tank, an air stone connected by tubing, to a reservoir was secured to the substrate. The reservoir was filled with half a liter of water from a tank (volume metal horse trough (237.5 x 86.4 x 60.1 cm) with 27 predatory largemouth bass *Micropterus salmoides*. Previous work has shown that individual crayfish react to aversive odours when making decisions whether to forage and use shelters (Jurcak and Moore, 2014). Next to the air stone, a 3 x 3 cm capsule of fish gelatin served as the food stimulus. The fish gelatin was made from 46 grams of homogenized canned sardines, 600 ml of boiling water and 28 grams of Knox's unflavored gelatin (Wolf and Moore, 2004). The liquid mixture was poured into separate circular 3 x 3 x 2 cm plastic bottle caps and refrigerated for twelve hours to allow the gelatin mixture to set (Lahman et al., 2015). An overhead camera, SONY Handycam HDR-CX405, recorded the 15 minute duration of the interaction. All procedures for the housing and care of the bass followed approved protocols (687097-4 [BGSU] and PRO00004591 [Michigan]).

Protocol

The crayfish was placed in the tank under a 14 x 14 cm square cage made from egg crating and was allowed to acclimate with the food and odour from bass water. For the first eight minutes of the acclimation period, water from the predatory bass tank was introduced via the air

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stone. After eight minutes, the flow of water containing bass odour was stopped. Preliminary dye work showed that this was enough time for the bass odour to cover 50% of the tank, leaving the food partially covered in bass odour and the shelter free of bass water. After the acclimation period, the cage was removed and the individual had 15 minutes to explore the tank, eat or hide.

Behavioural Analysis

The behaviours analyzed were time moving, time in shelter, and the weight of food consumed (Table 1). Time spent moving was defined as the duration of the assay (s) that an individual spent translating position in space as opposed to simply moving chelae and any time the animal location did not change but was climbing the wall. The time (s) in shelter was defined as the duration where the entire carapace or tail or both of an individual was covered by the PVC pipe shelter. The weight of food consumed was defined as the amount of food (g) that an individual consumed during the duration of the assay. The weight of food consumed was measured by weighing the fish gelatin before, and after the assay.

Foraging under different conditions

Arena

To determine the effect of exposed areas on foraging behaviour, crayfish were placed into a Y-maze (112 x 48 x 16 cm), with two arms (72 x 24 x16 cm). This set up has been used in previous experiments for food choice tests (Jurcak et al., 2015). At one end of the y-maze, were two openings, one in the center of the wall of each arm allowed water to flow through the y-maze (Figure 1 C). At the other end of the y-maze were five 2 cm diameters holes, 14 cm from the bottom on the wall opposite the opening. Each hole contained one elbow attachment and a piece of Tygon tubing that allowed for tank drainage and facilitated water flow. Two reservoirs were situated 72 cm above the anterior end of the y maze, each attached to a piece of hosing that fed into each arm of the y maze. One arm of the maze was covered with a wooden plank to darken that side of the y-maze while the other arm remained uncovered. The plank creates an unexposed portion of the arena. Both arms had one piece of fish gelatin (described above) placed at the center of each arm so the only difference between arms was that one arm was exposed.

Protocol

One arm of the maze was randomly selected via coin toss to be the dark arm and subsequently was covered with plywood. The left arm was covered 13 times and the right arm

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was covered 12 times. An individual crayfish was placed into the maze at the end opposite the two arms and allowed to explore the whole arena. Qualitative personal observations showed that individuals explored the entirety of the tank during acclimation. After the acclimation period of 15 minutes, the water flowed through the arena. The individual was corralled and again placed at the end of the arena opposite the arms. Then two 3 x 3 x 2 cm containers of fish gelatin as a food source were placed 36 cm from the end of each arm of the arena. As soon as the food was placed into the arena, the crayfish was given 15 min to consume the food.

Behavioural Analysis

The behavioural measures examined in the foraging under different light conditions were duration of time spent moving (s), duration of time spent in the lighted or uncovered arm (s) and the weight of food consumed (g) (Table 1). The total time spent in each arm was defined as the duration that an individual's entire carapace was in the arm and was moving or stationary. To calculate the total time spent in arm, the time spent in the covered arm was deducted by the time spent in the uncovered arm. The time spent moving was the duration an individual moved in the uncovered arm and non-arm section of the maze and was measured in seconds (The time spent moving did not include the covered arm because the animal was not visible). The weight of food consumed was measured by weighing the capsules with fish gelatin before and after the assay.

Agonistic assays

Arena

To determine agonistic behaviour of a focal animal, crayfish were placed into an arena designed to force interactions with an opponent. To perform agonistic contests, two animals were placed into two separate quadrants of a 40 x 40 x 14 cm fight arena. Each quadrant was separated from the other by a 19 cm removable opaque wall. The wall enabled chemical and physical isolation of the individuals before the fight assay period. This procedure is identical to others used previously with crayfish (Bergman et al., 2003: Figure 1 D).

Protocol

All focal animals were size matched with an unfamiliar opponent that was within 10% of carapace size to minimize the effect of size differences on the outcome of the fight (Cook and Moore, 2008; Fero et al., 2007). Animals were placed in separate quadrants of the tank and allowed to acclimate for 15 minutes. After the acclimation period, the opaque wall in the middle

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was removed and crayfish were allowed to interact with their opponent for 15 minutes. After, animals were removed from the tank. The tank was then rinsed with stream water to eliminate any potentially lingering chemical cues. During agonistic assays, the fight was determined to begin when the individuals were within two body lengths of each other and either touching or approaching with threat displays (Table 1). The fight was determined to be over when the individuals were separated by at least two body lengths for at least fifteen seconds. During agonistic assays, there are multiple beginning and endings of agonistic interactions called bouts. Bouts are defined as one beginning and ending of one interaction during the assay. For purposes of this experiment, only the first bout was used in the event of multiple bouts. Within crayfish agonistic behaviour, bouts after the first bout reinforce dominance status and have different temporal dynamics (Huber et al., 2001).

Behavioural analysis

Fights were scored using a previously established ethogram (Table 2; Bergman et al., 2003; Bruski and Dunham, 1987; Cook and Moore, 2008; Fero et al., 2007). This ethogram was used to score intensity level of the fight with higher levels of intensities being more costly and thus more risky for an individual (Bergman and Moore, 2003: Table 2). Three behavioral measures were also used to assess agonistic interactions. The time (s) to initiate the fight was defined as the time from the removal of the wall to the first interaction between individuals. Duration of time (s) the fight spent at non-escalated levels and duration of time (s) the fight spent at escalated levels were defined as the total amount of time spent at intensity levels -2 to 3 and the total amount of time spent at intensity levels 4 to 5, respectively. The first interaction was defined as the focal animal approaching within two body lengths of the opponent and engaging in one of the defined agonistic behaviours (Bergman et al., 2003; Bruski and Dunham, 1987). The duration of the fight spent at non-escalated levels was defined as the total time the two crayfish engaged in behaviours at intensity levels 2 to 3 (Table 1). The duration of the fight spent at escalated levels was defined as the total time that two crayfish engaged in behaviours at intensity levels 4 to 5 (Table 2).

Tail flip Initiation Distance (TID)

Arena

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To determine the startle or escape behaviour individual crayfish were placed into an arena that was designed to present an aversive or threating stimulus to provoke the animal to flee. This behavioural assay has been used to test the boldness of individuals (Carter et al., 2010). The arena consisted of a 15 x 40 x 15 cm (L x W x H) Plexiglas tank. A plastic opaque track (15 x 40 cm) was placed on top of the tank to allow a wooden cart to move from one side to the other. A centimeter width strip was cut through the center of the cart track to allow for the penetration of a glass rod hanging from the cart. Attached to the glass rod was a line of three ping-pong balls (3.81 x 3.81 x 3.81 cm) glued together in a horizontal array to encompass the width of the tank. A string was tied to a hook at the front of the cart and was attached to a motor that pulled the cart forward at 15 cm/s. The wall furthest from the camera was covered with 1 cm wide vertical black and white stripes, which were used to measure motion, distance, and speed. Finally, at the far end of the tank (in relation to the ping-pong balls) was a gate that secured the crayfish at one end of the tank for acclimation (Figure 1 E).

Protocol

At the start of the assay, the tank was filled with 3.78 liters of stream water and the track with the cart was then placed on the end of the tank farthest away from the gated area. At the beginning of the assay, a crayfish was placed into the gated section and acclimated for 2 minutes. This assay is a test of startle/escape behavior therefore it can only interact with the aversive stimulus once. After the acclimation period the gate was raised and the individual was allowed to move into the test area of the tank. The assay began once an individual left the gated area. At that time the motor was activated to move the cart toward the crayfish. The assay was complete once the ping-pong ball contacted the crayfish or the crayfish tail flipped (backwards fleeing motion controlled by the tail of an individual) or retreated.

Behavioural analysis

This assay involved three separate behavioural measures to quantify the response to a threatening object. Tail flip initiation distance was defined as the distance (cm) between the rostrum of the crayfish and the closest edge of the stimulus (ping pong balls), when a tail flip began. Reaction distance was defined as the distance (cm) between the closest edge of the stimulus (ping pong balls) and the rostrum of the animal when the animal displayed a response to the stimulus (meral spread, movement backwards, or freezing). Retreat speed was defined as the

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speed (cm/s) at which an individual retreated by walking or the speed of the tail flip away from the stimulus (Table 1).

Ecological Relevance

Field study

Twenty-four hours after all laboratory assays were completed, an individual crayfish was placed into a field study. A crayfish was tethered to a brick via a 15 lb test fish line tied securely to a piece of Velcro. The other half of the Velcro was securely fashioned to the back of the crayfish. In addition, the Velcro, chelae of the crayfish, and any exposed carapace were covered in white correction fluid to facilitate visualization under water. All field experiments were performed in the Maple River near the Experimental Stream Research facility at UMBS. The Maple River is a third-order boreal stream fed from the surface water of a nearby lake (Douglas Lake). The stream contains natural predators (beaver, trout, bass, and snapping turtles), detritus and other food sources, as well as native populations of *Orconectes propinquus*. The exact field location was the same for all crayfish and a site was located near one bank that was approximately 1.5 m deep. The substrate of the field location contained a mixture of sand, cobble, and large rocks that could serve as shelter. An underwater camera (Versacam remote zoom lens camera and waterproof housing; Fuhrman Diversified, Seabrook, TX, USA) was secured in the middle of a quadpod. On the top of the quadpod, red lights were mounted to supply enough light to video record behavior at night. Individual crayfish tethered on the bricks were placed underwater in the center of the set up under the camera and left for 24 hours.

Protocol

Each individual was superglued to Velcro, which was connected to a brick by a 15 cm long segment of fishing line. The fishing line allowed for individual movement during trial while staying within the frame of the camera. Fishing line and Velcro system has been used to tether crayfish in previous experiments and the tether system was designed such that all appendages would remain free from entanglement. Under these conditions, crayfish exhibit similar behaviors to those animals that have not been tethered (Schneider and Moore, 2000; Ludington and Moore, 2017). The presence of the tether does restrict some expression of behaviors and limits the degree of freedom for movement. This limitations are necessary in order to keep the animal within the viewing field of the camera while allowing outside predators and competitors to

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engage the focal animal. The brick and crayfish combination was taken to the Maple River where it was placed in the center of the tripod setup with an underwater camera. The brick was placed in the center of the screen of the camera to allow for visibility of the crayfish and behaviours throughout the 24 hour period. Videos were analyzed for behavioural measures.

Behavioural Analysis

In total, there were ten behaviours that were recorded from the field assays. The ten behaviours measured: Digging, Agonism, Antagonism, Predator-Prey, Shelter, Exploration, Foraging, Struggle, Mating and Stationary (Table 3). These behaviours were measured because they encompassed all activities seen while analyzing the field videos. The overall time an individual spent completing these actions over the entire 24 h period was recorded. Descriptions of individual behaviors can be found below in Table 3.

Data Analysis

Laboratory Data

A total of twenty-five individuals went through all five behavioural assays. In preparation for PCA analysis and structural equation modeling, each individual laboratory behavioural response (e.g., tailflip distance, time spent moving, etc.) was divided by the largest value that was measured for the population. This procedure resulted in a proportion for each response for each of the 15 different measures. Thus, all the measures were normalized to values between zero and 1. These proportions were loaded into an array that consisted of behavioural response on one axis of the array and individual animal on the other axis of the array.

Field Data

Similarly, each of the behavioural times for the field behaviours were normalized to the total time period of observations. Each field assay had a slight variation on the total time of observation (mean 23.6 ± 0.2 hours) resulting in proportions of time each field animal spent performing specific behaviours.

Statistical Analysis

PCA

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412 A total of 25 crayfish were used for statistical modeling. Statistical analysis of both
413 laboratory and field data followed procedures outlined in Dingemanse and Dochterman (2013)
414 and Dingemanse et al., (2007) for the PCA and multiple linear regression models. Structural
415 equation modeling, as outlined by Dingemanse et al., (2010), followed these first two analyses.
416 An initial PCA was performed for the laboratory data alone, the field data alone, and a combined
417 data set using the laboratory measures as active variables and field data as quantitative
418 supplementary variables. All PCAs were followed by a varimax rotation (Tabachnick and Fidell,
419 2001). Individual animals appeared only once within each field or laboratory assay. The results
420 of the PCAs were used to determine any possible a priori hypotheses of laboratory and/or field
421 behaviour. All PCAs were performed in Statistica ver 13.2. For the PCA analysis, the field
422 behaviors of mating and antagonism were dropped from the analysis because only 3 animals
423 exhibited mating behavior and only 5 animals exhibited any agonism in the field.
424
425 *General Multiple Linear Regressions*
426 Following the PCA analysis, general linear multiple regression models were performed
427 using the laboratory data to predict field behaviour. Laboratory and behavioural proportions were
428 z-transformed before loading into the multiple regression models to remove differences in mean
429 variance across the different laboratory assays (Dingemanse et al., 2010). Possible models were
430 identified using the 'dredge' script in the R package 'MuMIn' (Barton, 2015). Within the script,
431 an initial 'global model' was constructed for each field behaviour and all 15 lab variables as
432 predictors. The global model was subset to allow anywhere from zero (null model) to eight
433 laboratory variables for prediction of field behaviours. Models were identified and ranked
434 according to AIC, ΔAIC, R2, and model weight. Statistical significance of the differences in the
435 models was measured by using Akaike's information criterion (AIC). AIC values were
436 calculated from model discrepancies estimated by a maximum likelihood value using a
437 bootstrapping method (Bollen-Stine bootstrapping with 1,000 iterations). As Dingemanse et al.
438 (2010), significant models were determined based on the AIC of the model compared against the
439 subsequent model using AIC values AIC value (ΔAIC). If these values are greater than two, this
440 suggests less support for that combination of laboratory factors in predicting field behaviour
441 (Burnham and Anderson, 2002). Important to note is that ΔAIC values greater than two only
442 suggest less support and do not falsify the model (Burnham and Anderson, 2002). The ranked

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output models were then compared to *a priori* hypotheses identified in the PCA analysis. Significant models identified from the PCA and best fit general linear multiple regressions (ΔAIC below 2) were then used to construct structural equation models of observed field behaviours.

Structural Equation Modeling

The model results of the multiple regression models were used to create a priori hypotheses for the structural equation modeling (Dingemanse et al., 2010). Not all laboratory behaviours were considered for structural equation modeling due to non-significant results of PCA and regression analysis. Similarly, not all field behaviours had models with sufficient support to be included in SEM. The details for constructing and testing the SEM are found elsewhere (Dingemanse et al., 2010). Because of the large number of field behaviours (10), only structural models with significant predictive power from lab variables based on results in the linear regression were run. Thus, null models where no structure existed (as an *a priori* prediction from the multiple regressions) were not included in this analysis. As such, R2 from the SEM was used as a measure of explanatory power of the models instead of the suggested Dx (Dingemanse et al., 2010). These R2 can be used similarly to the R2 from regressions in a confirmatory manner for the model (Tabachnick and Fiddell, 2001). To verify differences between models in their fits to the causal relatedness of laboratory and field behaviours, the SEMs containing different regressions used to predict identical field behaviour were tested using a standard approach to compare regression coefficients (Zar, 1999). For example, one structural model could have laboratory assays 1, 2, 3, and 4 predicting field behaviours 1 and 2, whereas a second model could have laboratory assays 2, 4, 5, and 7 to predict field behaviours 1 and 2. Comparing SEM models with different regression coefficients allows the statistical validation of which laboratory assays are best predictors of similarly related field behaviours. Using a pooled standard error for each regression to create a ratio between the two models allows the construction of a t value to determine significance between two models (Zar, 1999). Structural equation models were conducted using the 'lavaan' R statistical package (Rosseel, 2012) and graphics of each model were constructed in semPlot using the semPaths function (Epskamp, 2014). Statistical comparisons between SEM models were performed in 'R' using the ANOVA

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function (R core team 2015) and are described in terms of AIC selection criteria listed in Table 4. Each SEM was Bollen-Stine bootstrapped with 1,000 iterations.

Results

The normalized results from both the laboratory and field data can be found in Appendix A and the loadings for the PCA tables can be found in Appendix B..

PCA Results

The results from the PCA on laboratory behaviors showed that the first two factors explained 43% of the variance in the laboratory assays (Figure 2 A, B). The third axis provided an additional 14% of explanatory power to the correlations. Factor 1 appears to describe an axis of activity, where more negative values are associated with less goal directed behaviours (using shelter, eating, or moving toward food) and the more positive scores correspond to goal directed movement (retreat and fighting). Axis 2 shows almost exclusively positive values, although consumption, reaction to the tail flip device, and walking against the wall are more negative. The axis for factor 3 shows a larger spread across the axis with fighting behaviours falling out more positively (Figure 2 B).

In a similar fashion, the PCA on field behaviours showed explanatory powers of 29% (axis 1), 20% (axis 2), and 17% (axis 3: Figure 2 A and B). Axis 1 exhibited more stationary behaviours on the positive side of the axis and moving behaviors on the negative side. Axis 2 appears to separate on goal-directed behaviours (positive values) and exploratory behaviours (negative values). Finally, axis 3 was associated with activity. Combining the field and laboratory behaviours on a single plot shows groupings of laboratory and field behaviours on the lower half of axis 2 and some disparate groups of behaviours on axis 1 and 3 (Figure 2 A and B).

GLMR results

The results from the general linear multiple regression models (GLMR) showed significant models for several laboratory and field variables (Table 4). The results from the GLMR include the results only for single field components and those laboratory behaviours that significantly explain the field behaviour. Table 4 is organized to only show top models based on the AIC criteria and models that had ΔAIC of greater than 2 are not shown (Dingemanse et al., 2010). Most notably, the field behaviour of “exploration” was significantly correlated with the

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laboratory behaviours of “escalated fights”, “moving for food”, “shelter use” and “against wall”. Similarly, “digging” was significantly correlated with “moving in maze”, “reaction”, “shelter use” and “escalated behavior” while “foraging” in the field was correlated with “escalated fights” and “moving for food”, “non-escalated behavior”, and “shelter use”. All other correlations are presented in Table 4. Field behaviours were subsequently grouped, based on overlapping correlated laboratory behaviours, for SEM analysis. For example, “exploration”, “digging”, and “foraging” were placed within one structural equation model that used “against wall”, “escalated behavior”, “movement for food”, “shelter use”, “movement in maze”, “reaction”, and “non-escalated behavior”” as predictors. This structure is called a module and is a set of related behavioural phenotypes that is demonstrated by which laboratory behaviours explain the field behaviour. Based on the output of the GLMR, two different structures were chosen for subsequent SEM modeling: Fighting behaviour (agonism and struggle), and searching (exploration, digging, and foraging).

SEM Modeling results

Results from the structural equation modeling produced two different potential modules connecting the laboratory behavioural assays with the field assays (Table 5). The first potential module can be thought of as a broad structure that might include the three field behaviours, Exploration-Digging-Foraging which can be explained by the laboratory assays of against wall, escalated behavior, movement for food, shelter use, movement in maze, reaction, and non-escalated behavior (Table 5: Figure 3). The second module structure connects the field behaviours of Agonism-Struggle with the laboratory assays of escalated behavior, against wall, shelter use, moving, and percentage of tank covered (Table 5: Figure 4).

Discussion

The results from this study demonstrated three distinct findings for correlated behavioural phenotypes in the crayfish, *Orconectes virilis*. First, correlate phenotype structures exist that can be elucidated from laboratory studies to predict behaviours in the field under more complex settings (Figure 3 and 4). This study found two different phenotype structures that explained five different field behaviours. These structures appear to correlate well with behavioural correlates described in previous work from which our definitions of modules and behavioural axes were taken (Mazué et al. 2015; Réale et al. 2007). The first module can be defined along the

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established exploration-avoidance behavioural axis and coupled with the laboratory behaviours associated with movement against wall, escalated behavior, movement for food, shelter use, movement in maze, reaction, non-escalated behavior) (Figure 3 and Table 5). These behaviors together are linked with the field behaviors of exploration, digging, and foraging. As such, these behaviors are related to some aspect of a non-goal directed movement throughout the environment. This module has been a common result across a wide array of species and conditions (Dingemanse et al., 2003; Dingemanse et al., 2002). Regarding our hypotheses, we did find some behavioural phenotypes that were correlated across the laboratory tests, but these correlations did not match our predictions for those field behaviours. With the behavioural modules found in these studies, only the classic bold-shy continuum seemed to be correlated both in the lab and the field. Finally, the only correlations that were matched by our *a priori* predictions were the individuals with high levels of aggression in the laboratory also had high levels of aggression in the field.

The second module is related to the boldness-shy behavioural axis and involves a wide diversity of laboratory behaviours (escalated behavior, against wall, shelter use, moving, percent tank covered) (Figure 4 and Table 5). Together these laboratory behaviors are predictive of the time spent in agonism or struggling found in the field behaviors. This module appears to line up with the classic bold-shy behavioural syndrome tested on many different organisms, where aggression and boldness are linked (Azevedo and Young, 2006; Holbrooket al., 2014; Wilson et al., 1993). Certainly, aggression has been connected to boldness in different model species (Carter et al., 2010) and is used to explain behaviors observed in the lab to those in nature (Sneddon, 2003). Crayfish engage in agonistic contests over resources in both laboratory and field conditions (Bergman and Moore, 2003; Bergman et al., 2003; Martin and Moore, 2007; Wofford, Earley and Moore, 2015). Given the prevalence of aggressive interactions in this organism, it is not surprising to find a module that aligns laboratory assays with field aggression.

The second overall finding from these results demonstrated that some behaviours, from tests designed with specific behavioural phenotypes in mind, were correlated to each other, but unconnected to the behaviour performed in complex natural situations (Figure 2). For example, the initiation of agonistic contests and time spent in non-escalated behaviours are key factors in determining the outcome of contests and hierarchy status (Moore, 2007). Yet, these behavioural factors in a laboratory setting were absent from any correlations of agonism and aggressiveness

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found in the field setting. In a striking contrast, several laboratory behavioural results related to foraging were completely absent from any of the structural models that predicted foraging in the field (Table 4). Prior to laboratory tests, crayfish had access to detritus albeit of smaller sizes and less variability than what was available in the field tests. In addition, the laboratory tests were run with protein based food sources as opposed to detrital food sources. The sudden increase in detrital abundance and the change in nutritional quality in the field might explain some of these results. These results indicated that, despite the well-used design of the behavioural assays, the simplicity of the laboratory setting is not predictive of a similar behaviour in the field.

Third and finally, not all of the field behaviour was present in the models based on the laboratory assays despite the diversity of different laboratory designs (Figure 2 and Table 4). Three important field behaviours (shelter use, antagonism, and stationary behaviours) were absent from any of the structural equations models that were significant. Similar to the conclusions above, these field behaviours probably arise from a more complex array of sensory stimuli (sensory landscape) that, in turn, requires complex neural processing (Cronin and Johnson 2014; Moore and Crimaldi 2004). The simplicity of laboratory conditions and the lack of a realistic sensory landscape may not provide suitable range of behavioural responses that are evoked under field conditions.

Connecting the field and lab assays

A more general conclusion from this work indicated that potential behavioral correlates connect behaviours observed from lab assays and behaviours observed in natural field settings. Still, certain laboratory behaviours were not good predictors of any field behaviours observed. The exploration assay and contradicting cues did not correlate with any behaviours seen in the field setting, which is contrary to expectations (Stamps and Groothuis, 2010). In addition, some field behaviors were not correlated with, or explained by any of the laboratory behaviours, such as mating, remaining stationary and antagonism. These field variables were taken out of the analysis because a minority of the individuals exhibited these behaviors. Clearly, some of the laboratory assays elucidated phenotypes that were absent in the field, which indicated that caution should be taken in attempting to derive the presence of field-based phenotypes from purely laboratory studies.

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Many current studies of behavioral phenotypes have called for the need to relate them to fitness consequences (Sih et al. 2015; McGlothlin et al., 2010; Wolf and Weissing 2012; Smith and Blumstein 2008). Different behavioral phenotypes have had consequences for mate selection (Godin and Dugatkin 1996), the survivability in predator - prey interactions (Lima and Dill 1990; Milinski et al. 1997), parental care (Budaev et al. 1999), and dispersal (Dingemanse and Réale 2005; Sih et al. 2004). Still, based on the results from this study, caution should be taken to extend some phenotypes discovered purely from laboratory studies to fitness consequences in field settings. Certainly, the contextual plasticity of laboratory sensory conditions is more simple than natural conditions (Stamps, 2016) and an approach where laboratory studies depend upon a multi-dimensional approach to context may be critical (Westneat et al., 2011, Dosmann and Mateo, 2014; Westneat et al., 2015).

Contextual plasticity could explain the existence of modules in some of the field assays. If some taxa, such as crayfish, have limited contextual plasticity, phenotypes may arise under certain ecological conditions such as a limited resource distribution or certain level of predation threat (Coleman and Wilson, 1998; Dingemanse et al., 2007; Dingemanse et al. 2010). Testing populations that inhabit environments with different levels of predation threats or even different distributions of sensory landscapes may produce a different outcome (Biro et al. 2004). Within crayfish, recent work has shown that even a simple environmental influence, such as flow (which subsequently structures olfactory signals), produces different behavioral patterns in foraging assays (Moore et al., 2015; Jurcak and Moore, 2016). Yet, even these studies measured behavioral patterns under the limited complexity of laboratory settings.

Nervous systems can be sculpted by previous experiences even on the scale of days (Yeh et al., 1996; Yeh et al., 1997). Developmental plasticity and the resulting behavioral phenotype are based in the underlying neuroarchitecture and functionality of the synaptic connections as it is altered by environmental conditions. The plasticity of nervous system may be a possible mechanism that explains the existence of similar behavioral responses across the varied contexts seen in this study. The diverse array of sensory stimuli in space and over time (sensory landscape and those concomitant experiences) under which organisms develop could be critical in determining not only where an individual's behaviour falls on a spectrum, but could also determine which behavioral phenotypes are even present within a population.

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CONCLUSION:

This study has demonstrated that some laboratory behaviours are correlated to behaviours seen under realistic environmental conditions. In addition, some field behaviours are not explained by their laboratory counterparts and some laboratory behaviours are unconnected to behaviours elicited under natural conditions. Certainly, caution must be taken when extrapolating behavioural phenotypes from the more simplistic laboratory conditions to the complex environmental of natural field conditions.

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831 TABLES

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832 Table 1. Descriptions of the 15 behavioural measures for each of the lab assays analyzed
833 in the videos listed in groups of three below. The assay number refers to the assay where the
834 behavioural measure was used. The numbers and corresponding assay for this table are: 1 TID, 2
835 Exploration, 3 Fighting, 4 Foraging in different conditions and 5 Foraging with conflicting cues.

Assay	Behavioural measurements	Description
1	TID	The distance at which an individual initiates tail flipping from the object (could be 0). Measurement is from the edge of the ping pong ball to the tip of the chelae of the focal animal the moment before the animal tail flips
1	Retreating speed	The speed at which an individual tail flips measured by the distance traveled in the first second of the tail flip, measured by the distance the animal travels during the tail flip until it hits the ground after one second. And the average speed of the animal retreating but is not retreating with a tail flip, measured by the distance the animal retreated in the first five seconds divided by the distance traveled
1	Reaction Distance	The distance at which and individual reacts behaviourally (increased antennae flicking, backing away, change in body position) that is not a tail flip after the cart has started moving. Measured from the edge of the ping-pong ball to the tip of the chelae using the back wall as the reference.
2	Time spent touching wall	The time at which an individual crayfish was within ½ of a body length from any wall
2	Time spent exploring	The amount of time during the assay that the animal was moving its entire body and changing the location of the body in the tank
2	Percent tank covered	The amount of surface area of the tank the crayfish covered
3	Time spent at 2-3	The amount of time at which the focal animal spends at the intensity levels of 2 and 3 from the ethogram
3	Time spent at 4-5	The amount of time at which the focal animal spends at the intensity levels of 4 and 5 from the ethogram
3	Time to start	The time at which the first bout in the video begins after the wall is removed. Beginning of bout is indicated by physical attack or aggressive position towards opponent within one and a half body lengths of each other
4	Food consumed (maze)	The weight of the food measured in grams, which were consumed during the assay. Initial mass of dish was subtracted by the ending mass of the dish
4	Time in arm	The amount of time during the assay that an individual's entire body was in the light arm or the dark arm.

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4	Time spent moving in maze	The amount of time during the assay that the animal was moving its entire body and changing the location of the body in the tank
5	Food consumed (conflict)	The weight of the food measured in grams that were consumed during the assay. Initial mass of dish was subtracted by the ending mass of the dish
5	Time in shelter	The amount of time that the animals entire section of carapace, tail or both, is covered by the shelter
5	Time spent moving toward food	The amount of time during the assay that the animal was moving its entire body to change location or rotate the body in the tank and is not in the shelter. This included any time the animal location does not change but was climbing the wall.

836

837 Table 2. Ethogram of agonistic behaviours based on level of aggression exhibited with
838 higher numbers indicating more aggressive behaviours.

Intensity Levels	Descriptions
-2	Tailflip away from the opponent or fast retreat
-1	Slowly back away from opponent
0	Ignore opponent with no response or threat display
1	Approach without a threat display
2	Approach with threat display using meral spread and/or antennal whip
3	Initial claw use by boxing, pushing, or touching with closed claws
4	Active claw use by grabbing opponent with open claws
5	Unrestrained fighting by grasping and pulling opponent's claws or appendages

839

840 Table 3. Descriptions of the behavioural measures from the field assay analyzed during
841 the video. Each behaviour seen visually during the videos is represented below.

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Exploration	The amount of time at which the focal animal is moving its entire body to change location or rotate body, is not in any shelters, and is not foraging
Agonism	The amount of time the focal animal spends fighting other crayfish
Antagonism	The amount of time the focal animal spends fighting other species that are not crayfish
Predator-Prey	The time during which a predator is closer than a body length (of the focal animal) away from the focal animal including any physical interaction the crayfish has with the predator
Shelter	The amount of time which the focal animal's carapace, tail or both are covered by a rock. And the time at which the crayfish is up against the rock or brick and curled up with chelae close to body and not moving except for rearranging the body. Time spent digging around shelter to create more shelter
Forage	The time which the focal animal is moving or not moving but is moving front two swimming legs near the front of the body, as well as actually eating
Stationary	The time which the focal animal is not moving the entire body, and legs are motionless
Mating	The time which the focal animal and other animal have claws locked together and bodies together in mating position
Digging	The time in which the focal animal is using chelae or its entire body to push sand, or carry it and displace sand from its original location
Struggle	An individual was considered struggling when it was attempting to move beyond the length of the tether.

Table 4. Top multiple regression models with ΔAICc below 2. Colors denote groupings for structural equation model construction.

Field Variable	Lab Variables	Adjusted R^2	F	AICc	ΔAIC	Model Weight
Exploration	Against Wall + Escalated + Moving for Food + Shelter Used	0.63	11.07	57.40	0.00	0.387
	Escalated + Moving for Food + Moving in Maze + Non escalated	0.60	10.09	58.97	1.57	0.176
Digging	Escalated + Moving in Maze + Reaction + Shelter Used	0.500	7.00	64.70	0.00	0.0799
	Escalated + Moving for Food +	0.49	6.74	65.24	0.538	0.061

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	Moving in Maze + Shelter Used					
	Moving in Maze + Reaction + Retreat Speed + Time Eating	0.46	6.08	66.69	1.99	0.030
Foraging	Escalated + Moving for Food + Non escalated + Shelter Used	0.48	6.49	65.79	0.00	0.110
Agonism	Against Wall + Escalated + Moving + Tank Covered	0.71	15.62	51.17	0.00	0.209
	Against Wall + Moving for food + Shelter Used + Tank Covered	0.69	14.26	52.87	1.69	0.089
	Against Wall + Moving + Retreat Speed +Tank Covered	0.69	14.16	52.99	1.82	0.084
Struggle	Escalated + Moving + Shelter Used + Tank Covered	0.74	18.20	48.22	0.00	0.867
Predator Interaction	Moving in Maze + Non Escalated + Time Eating	0.30	4.49	70.68	0.00	0.030
	Initiation + Moving in Maze + Non Escalated + Time Eating	0.35	4.33	70.99	0.32	0.026
	Moving for Food + Moving in Maze + Non Escalated + Time Eating	0..5	4.22	71.28	0.61	0.022
	Moving in Maze + Non Escalated + Reaction + Time Eating	0.34	4.09	71.64	0.96	0.019
	Against Wall + Moving in Maze + Non Escalated +Time Eating	0.32	3.94	72.06	1.38	0.015
	Initiation + Non Escalated +	0.20	4.00	72.16	1.49	0.014
	Moving in Maze + Time Eating	0.19	3.85	72.42	1.73	0.013

Table 5. SEM models of field behaviours using results from multiple regression models. SEM Bollen-Stine bootstrapping technique was conducted using 1,000 iterations in the R 'lavaan' package.

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Field Variable	Field behaviour R-square	SEM Bootstrap p-value	AIC
Exploration-Digging-Foraging	Exploration: 0.71;Digging: 0.34; Foraging: 0.46	0.15	668.02
Agonism-Struggle	Agonism: 0.78; Struggle: 0.76	0.26	420.31

FIGURE LEGENDS

Figure 1. Exploration. (Top view) Four walls faced into the center of the tank. Individuals were acclimated in the center of the tank (A). Foraging with conflicting cues. (Top view) A half PVC pipe in the top right corner acted as a shelter. In the center was a container of fish gelatin acting as an attractive cue and an air stone attached to a reservoir that introduced water from a predatory bass tank acting as an aversive cue (B). Light dark foraging. (Top view) A y-maze with food located in the center of each arm. One arm was randomly covered preventing light and acting as an unexposed area (C). Fighting arena. (Top view) The walls in the center were removable to allow separated acclimation, and then lifted to allow for interaction. The drawing shows four quadrants as this tank was taken from previous social experiments where two fights occurred simultaneously. In this experiment, only half of the tank was used as only one fight was run each time. (D). Tail-flip Initiation Distance. (Side view) A moveable cart was placed along track on the top of the tank. Ping-Pong balls were attached to the cart and dragged through the tank as the cart moved acting as an aversive stimulus for crayfish to perform escaping behaviours. One centimeter wide black and white markings to use for distance on side wall (E).

Figure 2. PCA analysis for laboratory behavioural assays using normalized proportions (top row). Distribution of laboratory results with the first two axes which explains 43% of the variation in data (A) and the first and third axes (B). The third axes explains an additional 14%. PCA analysis for field behavioural assays using normalized proportions (middle row). Distribution of field results with the first two axes which explains 49% of the variation in data (A) and the first and third axes (B). The third axes explains an additional 17%. PCA analysis for the combined laboratory behavioural assays and field behavior using normalized proportions (bottom row).

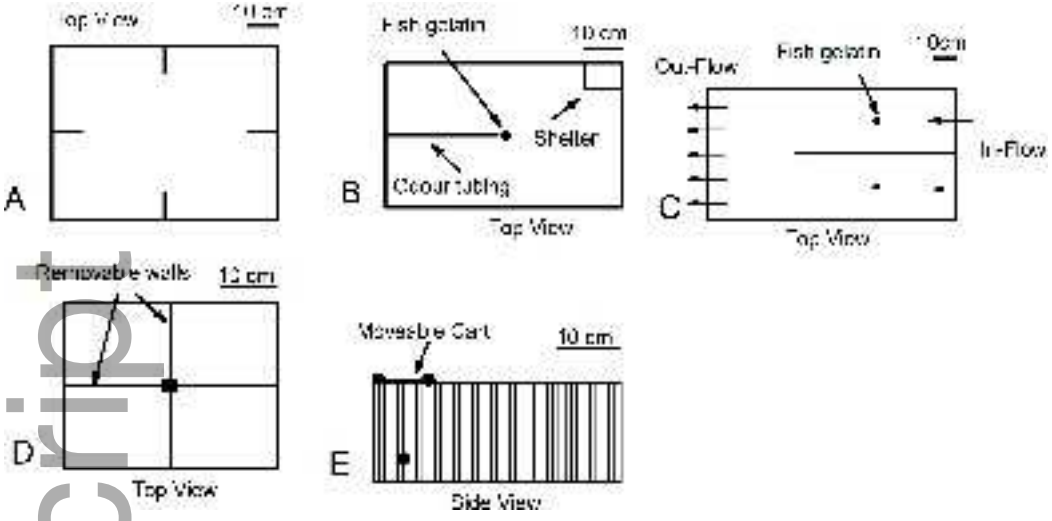
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875 Figure 3. The behavioural modules found from the behavioral matrix using laboratory assays to
876 predict field behavior for the exploration-avoidance module. The purple boxes represent field
877 behaviours and the gray boxes represent lab behaviours. The lines represent correlations between
878 behaviours with the number representing the strength of the correlation (the higher the number
879 the stronger the correlation), and whether the correlation is positive or negative.
880
881 Figure 4. The behavioural module found in lab, field and lab and field settings for the bold-shy
882 module. The meaning of the different numbers are explained in legend for Figure 3.

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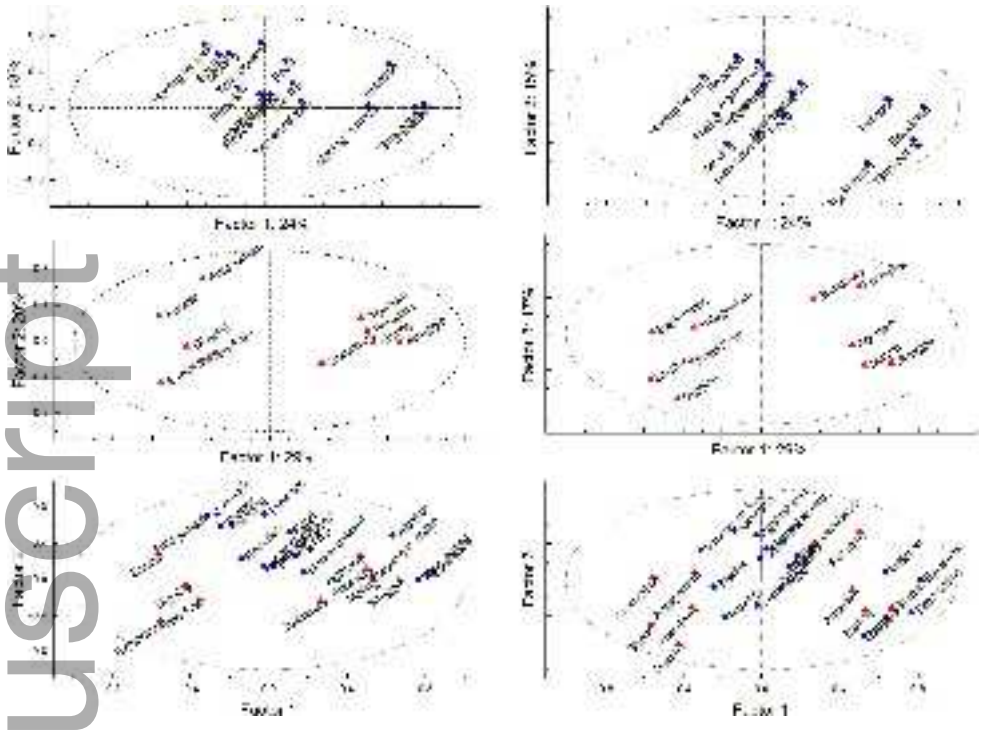


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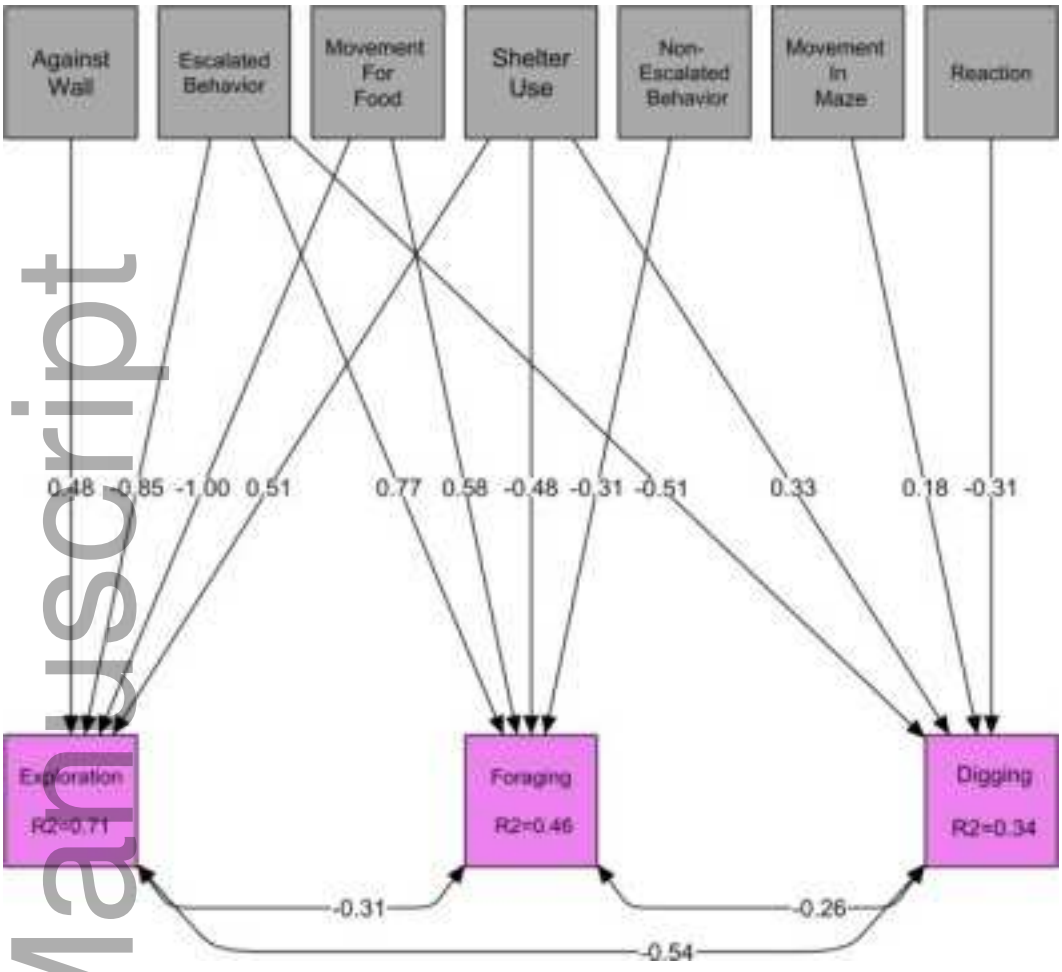
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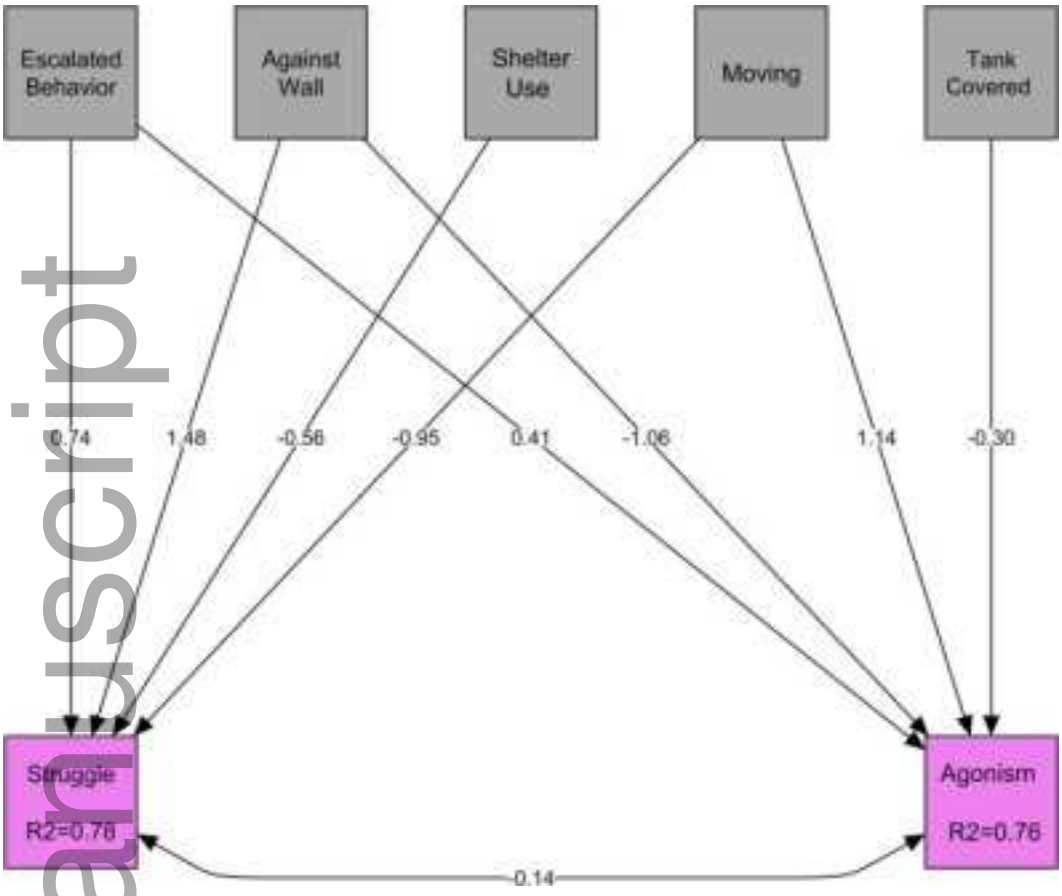
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