



Rate of movement of juvenile lemon sharks in a novel open field, are we measuring activity or reaction to novelty?



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Personality differences are widespread throughout the animal kingdom and can have important ecological and evolutionary consequences. Despite a rapidly increasing body of literature, large (marine) vertebrates remain underrepresented in personality research. Given their unique life history traits (e.g. slow growth rate, slow reproduction rate, long life span) and their pivotal role in ecosystem processes, this is an important gap in our current knowledge. Here we investigated consistency and plasticity in movement behaviour of wild juvenile lemon sharks, *Negaprion brevirostris*, by repeatedly subjecting sharks to open field tests. First, we investigated the presence of interindividual differences in movement behaviour in a novel open field. Second, we investigated the effect of trial repetition on movement behaviour to understand whether movement in a novel open field reflects a reaction to novelty, or general activity. Third, we estimated individual differences in habituation/sensitization rates over trial repetition and studied how the habituation rate was predicted by the initial movement rate. We found consistent individual differences in movement behaviour during the open field tests. Sharks showed habituation in movement behaviour (i.e. decrease) over repeated trials indicating that the movement behaviour during the first trials is a reaction to novelty, and not general activity. Individuals, however, differed in their rate of habituation (i.e. plasticity) and this rate was negatively related to an individual's movement behaviour in the first open field trial. In addition to showing individual differences in consistency and plasticity in juvenile lemon sharks, our study emphasizes the importance of examining the validity of personality tests when adapting them to new species.

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The discovery that individuals can show consistent behavioural differences through time (i.e. animal personality; Biro & Stamps, 2008) has shifted the traditional view of individual variation from random noises to a biologically important phenomenon. Indeed, such differences in individual behaviour can have important ecological and evolutionary consequences (Sih, Bell, & Johnson, 2004; Wolf & Weissing, 2012) and enhance management programmes (Conrad, Weinersmith, Brodin, Saltz, & Sih, 2011; Mittelbach, Ballew, Kjelvik, & Fraser, 2014). As a result, research on animal personality is currently booming and knowledge is rapidly accumulating on a diversity of species (Gosling, 2001; Réale,

Reader, Sol, McDougall, & Dingemans, 2007; Sih, Bell, Johnson, & Ziemba, 2004). However, despite this rapid expansion much of our understanding comes from studies on captive animals that are easy to house and with a short life span (Archard & Braithwaite, 2010). This bias has led to an underrepresentation of large animals, especially large marine vertebrates which are usually characterized by slow growth rate and reproduction rate, long life span and a relatively high trophic position (Jennings, Pinnegar, Polunin, & Boon, 2001; Lewison, Crowder, Read, & Freeman, 2004; Romanuk, Hayward, & Hutchings, 2011; Stevens, Bonfil, Dulvy, & Walker, 2000). These characteristics make them both important to ecosystems processes and highly vulnerable to anthropogenic impact (Estes et al., 2011; Stevens et al., 2000). Furthermore, obtaining data on a wide variety of organisms with different life history and ecological conditions is warranted to understand the evolution of animal personality (Réale et al., 2007; Réale et al.,

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2010). Therefore, expanding personality research to animals with longer life spans is vital.

Advancing knowledge of large aquatic organisms is challenging because of logistical constraints (e.g. sample size, capture constraints, housing difficulties). However, novel technologies such as biotelemetry (radio and acoustic telemetry) and biologging (archival logger) devices now offer sophisticated means of evaluating the behaviour, spatial ecology, energetics and physiology of these animals in their natural environment (Cooke et al., 2004; Hussey et al., 2015; Krause et al., 2013). In recent years, applying these techniques has led to the discovery of individual variation in movement patterns, habitat use and feeding habits for various large marine animals, such as mammals, sharks, birds and reptiles (Hatase, Omuta, & Tsukamoto, 2007; Heithaus, Dill, Marshall, & Buhleier, 2002; Kuhn, Crocker, Tremblay, & Costa, 2009; Matich & Heithaus, 2015; Patrick et al., 2014; Rosenblatt & Heithaus, 2011; Tinker, Costa, Estes, & Wieringa, 2007; Vaudo et al., 2014). The observed individual differences, however, cannot easily be directly linked to personality due to the challenge of disentangling personality from various other factors (e.g. environmental or population differences). It is, therefore, pertinent to develop appropriate captive personality tests that complement these field data. Such experiments have been adapted successfully for sharks, identifying social personalities in catsharks, *Scyliorhinus canicula*, and showing the importance for their social structure of individual differences in the locomotion behaviour of juvenile lemon sharks, *Negaprion brevirostris* (Jacoby, Fear, Sims, & Croft, 2014; Wilson et al., 2015). However, the development of standardized tests to detect consistent individual differences in sharks' movements is still lacking. The 'open field test' has frequently been used to quantify consistent individual differences in movement and is, therefore, a promising candidate to investigate personality in sharks.

Developing and interpreting personality tests can be challenging (Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013) and open field tests are no exception. In their seminal study, Réale et al. (2007) proposed five distinct personality axes: aggressiveness, sociability, shyness–boldness, exploration–avoidance (also called neophilia) and activity. Within these axes, the behaviours during open field tests have mostly been interpreted as exploration (Conrad et al., 2011; Garamszegi, Markó, & Herczeg, 2013) but also as boldness (Toms, Echevarria, & Jouandot, 2010) and, when distance moved is recorded, as activity (Carter et al., 2013). Various methods can help to interpret behaviour during personality tests. Carter et al. (2013) discussed the use of convergent (i.e. different tests measure the same personality trait) and discriminant validity tests (i.e. different tests measure different personality traits). For example, in guppies, *Poecilia reticulata*, movement activity in an open field test was not correlated with activity in a nonstressful environment (Burns, 2008), suggesting that the open field test measures reaction to novelty and not general activity (i.e. discriminant validity). However, in this study (and for large vertebrates in general) performing multiple tests was logistically difficult.

Another method to verify a reaction to novelty is to repeatedly expose individuals to the same open field (Warren & Callaghan, 1976). If the observed behaviour is a reaction to novelty, it is expected to covary with the number of exposures (i.e. habituation and/or sensitization; Groves & Thompson, 1970). Thus, when facing logistical constraints, testing habituation and/or sensitization can be a viable alternative. In addition, several studies have demonstrated high individual variation in the strength and direction of the response change, with such variations being related to an individuals' personality (Mathot, Wright, Kempnaers, & Dingemanse, 2012). Personality-related differences in plasticity (also known as behavioural reaction norms) have gained attention because of their evolutionary and ecological significance

(Dingemanse, Kazem, Réale, & Wright, 2010; Dingemanse & Wolf, 2013; Martin & Réale, 2008; Mathot et al., 2012). Thus, when repeatedly exposing individuals to the open field, it is possible to also investigate the presence of individual differences in plasticity and its effect on repeatability.

In this study, we used the lemon shark, a common large coastal species in the western Atlantic whose biology, behaviour and ecology have been extensively studied (Guttridge et al., 2011; Guttridge et al., 2009; Sundström et al., 2001). At our study site in Bimini, Bahamas, juveniles (<4 years) use the mangrove-fringed shoreline which offers a shallow (<0.5 m depth) and protected habitat (Newman, Handy, & Gruber, 2007). Adjacent to the shorelines are deeper seagrass flats which older conspecifics (>120 cm total length) occupy during favourable tides to predate upon the juveniles (Guttridge et al., 2011; Morrissey & Gruber, 1993a). Despite having a home range close to the shoreline, some juvenile lemon sharks venture into these riskier habitats (Morrissey & Gruber, 1993b). Dibattista, Feldheim, Gruber, and Hendry (2007) demonstrated that sharks that were large at birth and fast growing had higher mortality rates than smaller, slower growing individuals. These findings, together with their ease of capture, abundance and robustness in captivity, make the lemon shark an excellent model species for cartilaginous fishes and large marine vertebrates to experimentally investigate individual differences in a novel open field.

Juvenile lemon sharks were observed on six occasions in an open field, with the following aims: (1) to test the repeatability of their rate of movement (ROM) to investigate the presence of interindividual differences in movement behaviour; (2) to test the variable ROM for habituation and/or sensitization with repeated exposure to the open field to understand whether the behaviour is a proxy for activity or for reaction to novelty; and (3) to investigate the presence of individual differences in the strength and/or direction of such a habituation/sensitization effect and how differences in these effects, in turn, relate to personality.

METHODS

Study Site and Experimental Set-up

This study was conducted in Bimini (20°–28°N, 72°–80°W), Bahamas, a chain of islands situated approximately 85 km east of the coast of Florida, U.S.A. In total, 28 juvenile lemon sharks (14 females and 14 males) were captured using gillnets (see Manire & Gruber, 1991 for details). Upon capture, each individual was measured for body size (mean precaudal length (PCL) \pm SD = 53.23 \pm 4.79 cm), sexed and equipped with a unique colour-coded tag (T-bar type, Floy Tag Inc, Seattle, WA, U.S.A.) for visual identification.

Sharks were housed in a large circular holding pen (10 m diameter) constructed just offshore in shallow (<1.5 m) sand bottom flats (see Guttridge et al., 2009 for details). Sharks were given a minimum of 2 days to acclimatize to captive conditions. During nonexperimental periods, sharks were fed to satiation every 3 days on a diet of fresh and frozen local fish (*Sphyræna barracuda*). During experimental periods, sharks were always fed the day before an observation day (see below).

Secured to the holding pen was a start box (semicircle; 1.5 m radius) that provided access to a rectangular (6 \times 12 m) open field split into 18 sectors (2 \times 2 m) by ground markers (Fig. 1). Sliding doors (manually operated) were used to control the movement of sharks between the three compartments, with an external exit channel attached to the test pen to facilitate the return of sharks after trial completion. Individuals had never been introduced to this pen before the trials. Behavioural observations were conducted from a 2 m high observation tower.

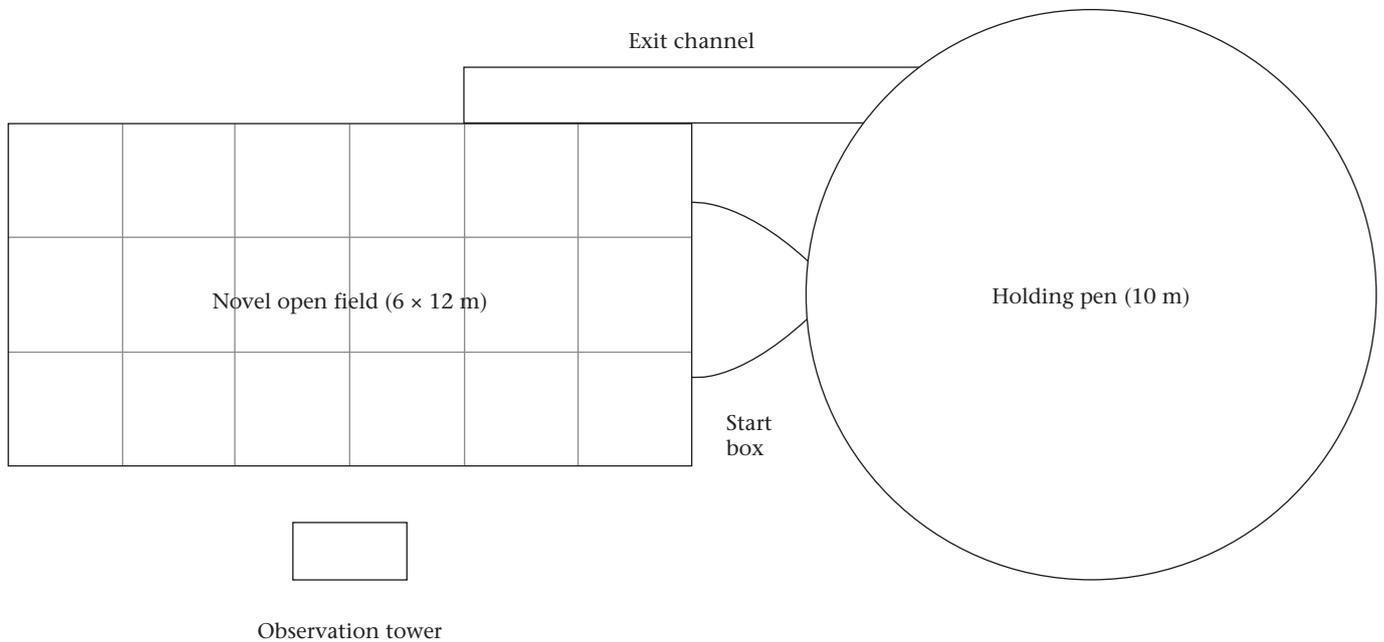


Figure 1. Schematic overview of the experimental set-up used for testing the movement behaviour of juvenile lemon sharks in a novel open field.

Experimental Procedure

Prior to the test, a predetermined shark was ushered into the start box. After 5 min, the sliding door was opened, allowing the shark to enter the test pen. The door to the start box was then gently closed. The shark was observed in the test pen for 10 min, during which the total number of sectors visited was recorded as a measure of the shark's ROM, as well as the total number of new sectors visited. Sharks were recorded as having entered a sector once their head and first dorsal fin crossed the demarcation line. Upon trial completion, the shark was ushered back to the holding pen via the exit channel. Each shark completed six trials, one every 2 days across a 12-day period. If observations from the tower were not possible due to inclement weather (e.g. rainstorms), sharks were still subject to the same procedures and these trials ($N = 7$, <5%) were treated as missing values. For two trials environmental values were not taken (see below). These trials were also excluded resulting in 159 trials for 28 individuals.

All trials were conducted at least 2 h after sunrise and before sunset to avoid luminosity biases. The exact timing of each trial was scheduled using tide predictor software (WXtide32: <http://www.wxtime32.com>), to minimize any potential effects of differences in water depth between trials (mean \pm SD = 55 \pm 5.5 cm) and to retain the same current direction (rising tide). Therefore, the number of animals tested per day was restricted to a maximum of three. By alternating two groups of the maximum three individuals each day, six individuals could be observed across a 12-day period. However, depending on the number of animals captured during gillnetting some of these periods contained fewer than six individuals. In total there were six of these 12-day periods. For each trial, percentage cloud cover, underwater visibility (using a Secchi disc) and wind speed were recorded and controlled for in the following analyses.

Data Analysis

Statistical analyses were only applied to ROM due to the very low variation in the total number of new sectors visited (the first

quartile and the median were the maximum: 18 sectors). ROM was square root transformed and checked for normality using a Shapiro test. All statistical procedures were performed in R v3.2.3 (R Core Team, 2015) using the lme4 package for mixed models analysis (Bates, Mächler, Bolker, & Walker, 2015). Significance levels were derived using the package lmerTest (Kuznetsova, Brockhoff, & Christensen, 2015) based on Satterthwaite's approximations.

Repeatability

To test for repeatability in ROM, a linear mixed model was used with ROM as response variable, shark ID as random factor and trial number (continuous: 1–6), time period (number of days since the start of the experiment; continuous: 1–75), body size, sex, wind speed, cloud cover, time in captivity (continuous: 2–33 days) and visibility as fixed effects. The significance of the random term was assessed using the function exactRLRT from the package RLRsim (Scheipl, Greven, & Küchenhoff, 2008) with 10 000 simulations. Finally, repeatability was calculated by dividing the individual variance by the total variance (individual plus residual variance). To test for a potential effect of habituation on repeatability, we performed similar analyses using subsets of the data: (1) trials 1 and 2, (2) trials 1 and 6 and (3) trials 5 and 6.

Individual variation in habituation/sensitization

To test for the significance of personality-related differences in habituation/sensitization, a random slope mixed model with the interaction between trial number and shark ID was compared to a similar model but with only shark ID as random term. A likelihood ratio was calculated between these two models and significance was assessed against a chi-square distribution of two degree of freedom. Finally, repeatability was calculated by dividing the variance explained by shark ID by the total variance of the random slope mixed model (shark ID, Residuals and Slope variance), using the same response variables as described above.

To improve the interpretation of our random slope mixed model analysis, we carried out a power analysis. We performed a similar analysis as described above using only trial number as a fixed effect.

The parameters obtained were used in the PAMM package (Martin, Nussey, Wilson, & Réale, 2011). We allowed the number of simulated individuals to vary between 20 and 100 (increments of 10), keeping the number of replicates fixed at six.

Owing to the lack of power (see Results), we used a second method to investigate the relationship between personality and differences in plasticity. The effect of trial number on ROM was linearly regressed for each individual and the slope was extracted as an estimate of an individual's habituation/sensitization rate. To investigate the relationship between ROM during trial 1 and this habituation/sensitization rate, a linear regression was used with habituation/sensitization rate as dependent variable and ROM scores from trial 1 as an independent variable. Body size, time period, time in captivity before trial 1 (continuous 2–22 days) and sex were included as fixed effects. Lastly, we investigated the relationship between ROM in trial 1 and habituation/sensitization rate using a Spearman rank correlation test.

Ethical Note

All procedures were approved by the Department of Marine Resources, Bahamas (Permit no: MAF/LIA/22). No sharks died during the course of the experiments and, after the experiment, sharks were released at their site of capture with their colour tag removed. Sharks were kept for a maximum of 40 days to limit any potential impact of captivity. All manipulations (e.g. size/sex determination and tagging) were performed within 5 min to minimize handling stress. No steps required anaesthetizing the animals as this would increase manipulation time, increasing the stress on the animal.

RESULTS

Repeatability

Juvenile lemon sharks showed consistent individual differences in their ROM when including all trials (repeatability = 0.28; restricted likelihood ratio test, restricted likelihood ratio = 16.3, $N = 28$, $P < 0.001$). ROM decreased with the number of trials showing an overall effect of habituation. Further, body size, cloud cover and time period significantly affected ROM; however, there were no significant effects of sex, wind speed, visibility or time in captivity (Table 1).

Interestingly, sharks showed consistent individual differences when trials 1 and 2 were compared, no consistent differences comparing trials 1 and 6 and a trend comparing trials 5 and 6 (trials 1 and 2: repeatability = 0.50; restricted likelihood ratio test, restricted likelihood ratio = 5.02, $N = 26$, $P = 0.01$; Fig. 2a; trials 1 and 6: repeatability = 0.03; restricted likelihood ratio test, restricted likelihood ratio = 0.01, $N = 26$, $P = 0.45$; Fig. 2b; trials 5

Table 1

Results of a linear mixed model testing the effects of several fixed effects on the rate of movement (ROM) in an open field over six repeated trials with individual as random factor

Variable	Estimates \pm SE	df	t	P
Intercept	11.00 \pm 1.02	24.27	10.82	<0.001
Trial number	-0.074 \pm 0.027	128.04	-2.75	<0.01
Body size	0.053 \pm 0.018	23.28	2.95	<0.01
Cloud cover	0.003 \pm 0.0015	139.02	2.01	0.046
Time period	0.0083 \pm 0.0035	26.01	2.33	0.028
Time in captivity	0.015 \pm 0.014	23.08	1.07	0.30
Visibility	-0.078 \pm 0.045	141.32	-1.75	0.083
Sex (male)	-0.088 \pm 0.183	22.93	-0.48	0.63
Wind speed	-0.0043 \pm 0.0073	135.58	-0.588	0.56

Bold values indicate P values below 0.05.

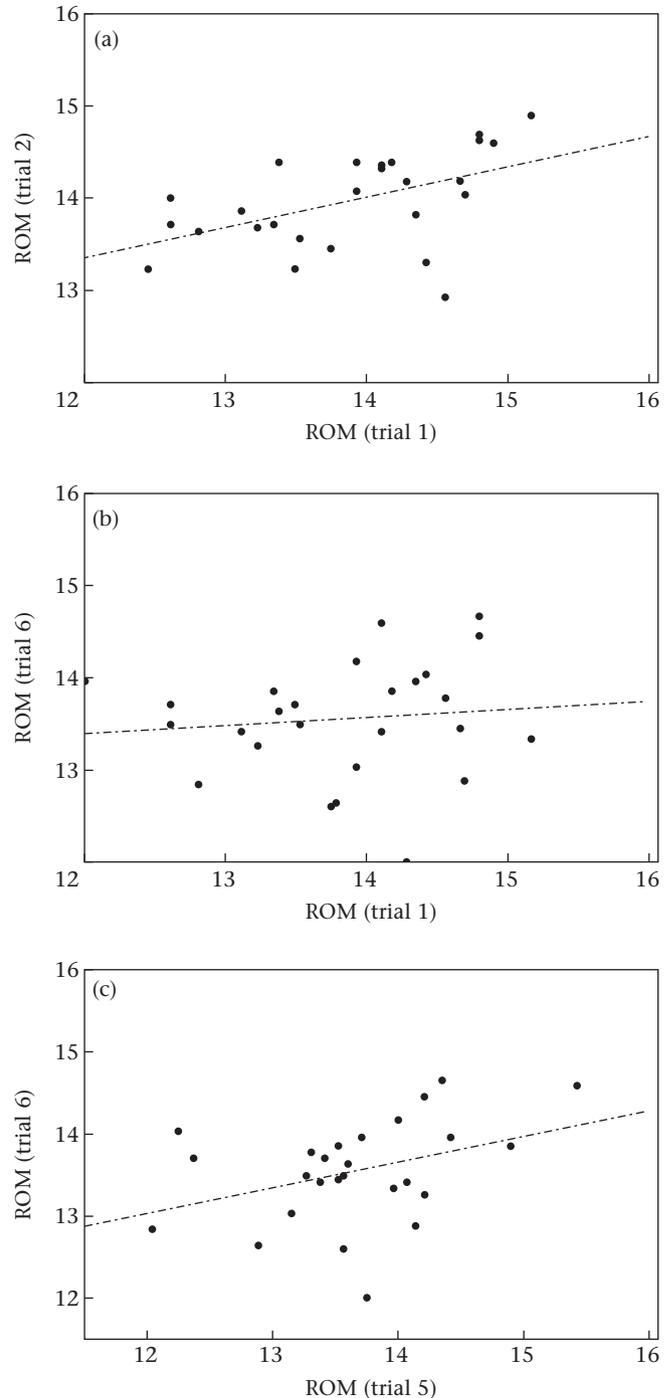


Figure 2. Comparison of lemon sharks' rate of movement (ROM) in (a) trials 1 and 2, (b) trials 1 and 6 and (c) trials 5 and 6. Square root transformed ROM values were used to draw the linear regression lines.

and 6: repeatability = 0.39; restricted likelihood ratio test, restricted likelihood ratio = 2.01, $N = 26$, $P = 0.08$; Fig. 2c).

Individual Variation in Habituation/Sensitization

The random slope mixed model analyses suggest that individuals did not differ significantly in their rate of habituation/sensitization (likelihood ratio test, likelihood ratio = 1.68, $N = 28$, $P = 0.42$) despite a high correlation estimate between individuals'

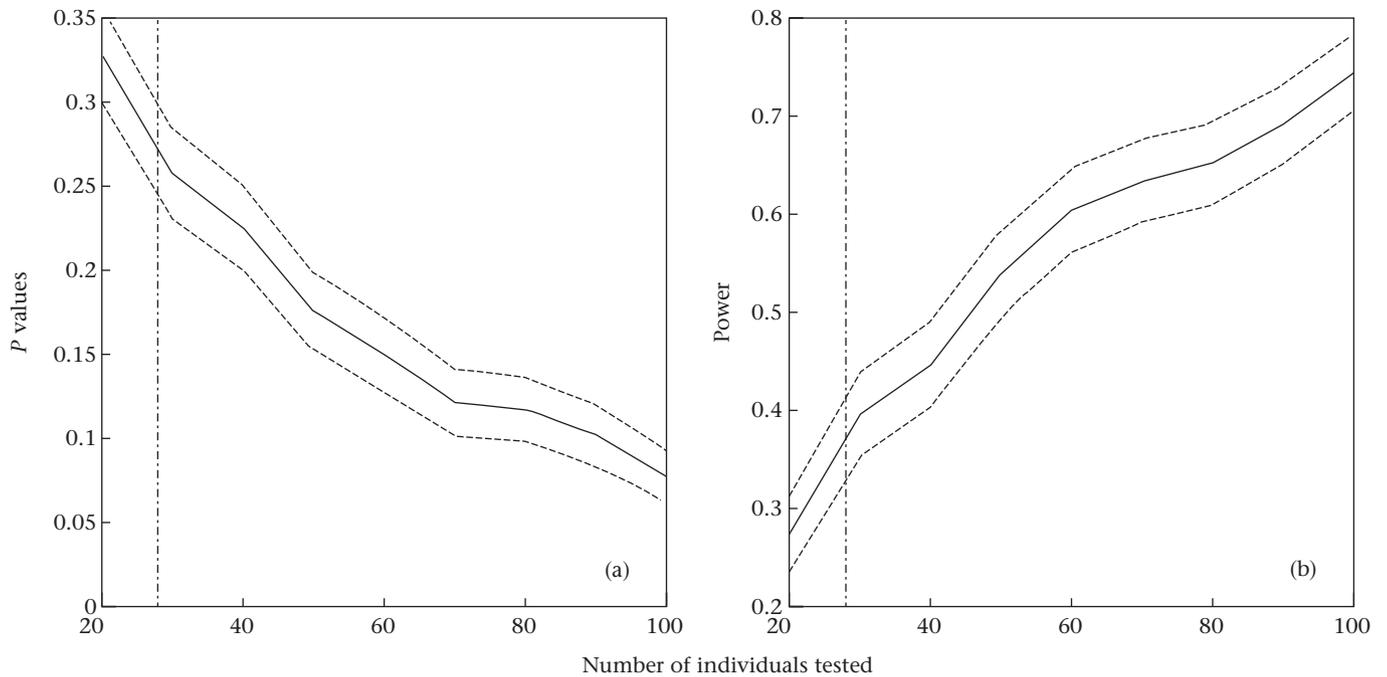


Figure 3. (a) Simulated P values and (b) power analysis to detect the significance of the random slope as a function of the number of individuals tested. This simulation was based on random slope mixed model results obtained in this study and with the number of replicates per individual fixed at six. Vertical lines represent the actual sample size ($N = 28$).

intercepts and slopes (correlation: -0.78). When individual variation in plasticity was accounted for, the repeatability score obtained was 0.51. We obtained similar results when only including trial number as response variable (likelihood ratio test, likelihood ratio = 5.51, $N = 28$, $P = 0.063$; correlation: -0.76). This lack of significance should, however, be taken with caution as additional simulations show that the power, obtained with our sample size, is rather low (Fig. 3).

Using the simpler linear regression analysis, we found that the habituation/sensitization rate covaried negatively with the increase in ROM during trial 1 (estimate \pm SE = -0.16 ± 0.035 ; Student t test: $t_{22} = -4.506$, $P < 0.001$; Fig. 4). Interestingly, sharks ranged from either a decrease, increase or no change in ROM over trial repetition. Furthermore, time period had a significant positive effect on the habituation/sensitization rate (estimate \pm SE = 0.003 ± 0.001 ; Student t test: $t_{22} = 2.702$, $P < 0.05$). There was no significant effect of time in captivity before trial 1, sex or body size (all $P > 0.05$). In addition, when using the Spearman rank test, we found a negative correlation between ROM during trial 1 and the habituation/sensitization rate (Spearman rank correlation: $r_s = -0.55$, $N = 28$, $P < 0.01$).

DISCUSSION

Our results show that juvenile lemon sharks have consistent individual differences in their rate of movement (ROM) when tested repeatedly in an open field. In addition, we found that ROM in a novel open field can be used as a proxy for reaction to novelty since a decrease (i.e. habituation) was observed with trial repetition (exposure to the same open field). Finally, we showed that although overall habituation was found with trial repetition, individuals differed in the way they changed (strength and direction) their ROM with repeated exposures. These changes in ROM covaried negatively with ROM during trial 1: individuals with a high ROM in trial 1 showed quick habituation, whereas individuals with a low ROM showed no change or even sensitization (i.e. increase in ROM).

Consistent individual differences in open field tests have been demonstrated in many taxa (e.g. Boon, Réale, & Boutin, 2007; Budaev, 1997; Burns, 2008; Dingemanse et al., 2012; Rodríguez-Prieto, Martín, & Fernández-Juricic, 2010; Verbeek, Drent, & Wiepkema, 1994) and this is the first demonstration in elasmobranchs. This is an important prerequisite for linking captive behaviour with field observations, paving the way for more in

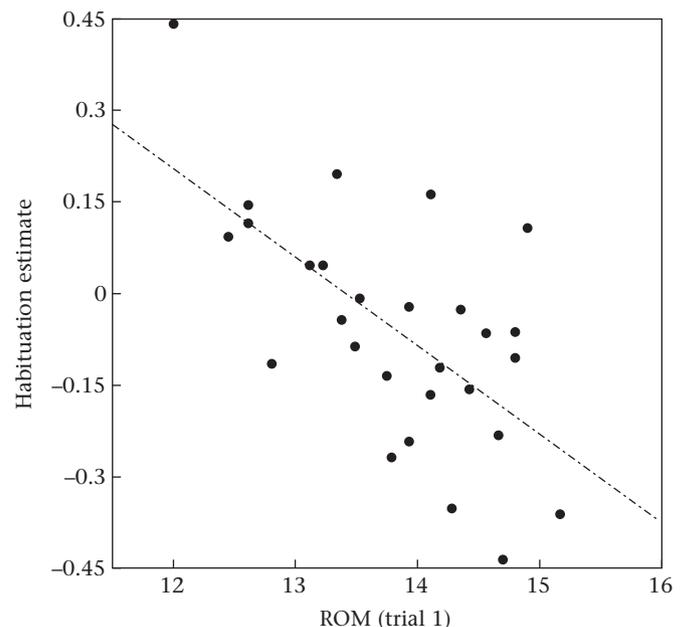


Figure 4. The rate of movement (ROM) during trial 1 in relation to the subsequent rate of habituation/sensitization. Negative values indicate habituation (i.e. decrease in movement activity); positive values indicate sensitization. Square root transformed ROM values were used for a linear regression.

depth exploration, such as the proximate and ultimate causes of these differences. An important future step is also to investigate consistency and plasticity of individual differences over longer time periods. We tested juvenile sharks and although personality can be long lasting, even across ontogenetic shifts (Wilson & Krause, 2012), personality is not always stable across the life span (Petelle, McCoy, Alejandro, Martin, & Blumstein, 2013). Overall, our study demonstrates the potential of the open field test for elasmobranchs and hopefully stimulates the use of this method to test and understand consistent individual differences in lemon sharks and other elasmobranchs.

As already noted, behaviours during open field tests can be interpreted differently. We observed a change in ROM with repeated exposures that we ascribe to novelty loss (Dingemans et al., 2012; Warren & Callaghan, 1976). Therefore, this behaviour most likely reflects a reaction to novelty and not solely general activity. Typically, the open field test is thought to measure exploration; however, some authors have interpreted it as a boldness test (Toms et al., 2010). Stress is likely to play a role in any experiment using novelty. For example, less explorative individuals are more likely to be stressed by exposure to the open field than more explorative ones. In such a scenario, it becomes very challenging to disentangle neophilia and boldness and this might also depend on the species studied (for instance its trophic level associated with the risk perceived from a novel environment). Not only is it unclear how novelty is perceived but also whether individuals habituate to the open field per se. Our open field test encompassed novelty in its shape, and sharks had never visited it before, but the environment did not differ markedly from the holding conditions. Therefore, one might question the strength of the novel environment stimulus and other interpretations should be also considered. First, the observed decrease in ROM could reflect habituation to the ushering and handling in general rather than the novel open field per se. Although we cannot exclude this possibility, we believe that handling stress was minimal: sharks were never directly handled, never entered the start box in an erratic way while being ushered, and were given 5 min before entering the test pen by themselves. Second, our results might be due to habituation to being in captivity. However, since time in captivity had no effect on ROM or habituation/sensitization rate we can probably rule out this possibility. Using a habituation/sensitization approach can be instrumental in guiding research in this and other species and has demonstrated that activity in a novel open field is not to be confounded with general activity. Future studies could produce validity tests (e.g. Beckmann & Biro, 2013; Burns, 2008; Carter et al., 2013; Dochtermann & Nelson, 2014) and pinpoint the exact stimuli to which sharks habituate.

Individuals varied in their change in ROM and these changes were negatively correlated with their first ROM scores. These analyses were performed following the logic under the reaction norm framework (Dingemans et al., 2010); our results suggest that elevation (ROM trial 1) and slope (change in ROM over trials) covary. Behavioural reaction norms are usually analysed with random slope mixed models (Dingemans et al., 2010). However, in our case the power was insufficient to allow this approach. Despite this low power, a strong negative correlation between intercept and slopes was still found. Furthermore, when controlling for personality-related differences in habituation/sensitization, the repeatability estimate increased to 0.51 (from 0.28). Therefore, instead of dismissing individual variation in plasticity, we used a method similar to Rodríguez-Prieto et al. (2010) to obtain estimates of the change in ROM with trial repetition. Doing this, we found a strong negative relation between ROM trial 1 and habituation estimates demonstrating a relationship between personality and plasticity (i.e. habituation/sensitization). This method is less accurate than the

random slope models (Martin & Pelletier, 2011) but, in our opinion, the presence of strong individual differences in habituation/sensitization rate which are related to personality cannot be dismissed. Sharks could only be kept for relatively short periods and observation time was constrained by external factors such as tide or light, constraints not uncommon for studies on aquatic megafauna. In such scenarios, it is important to conduct power simulations to avoid dismissing biologically important observations (Martin et al., 2011).

The repeatability estimate over all six trials (0.28) was substantially lower than when controlling for individual differences in plasticity (0.51) or when only the two first trials were included (0.50). Most likely, with increasing exposure the effect of individual differences in plasticity changed the ranks of individuals obtained in the first trial, as further evidenced by the loss of repeatability when only the first and last trials were considered. One alternative explanation might be a reduction of individual variances during habituation. Indeed, the loss of significance when only trials 5 and 6 were considered might indicate this. However, a trend was still apparent comparing trials 5 and 6 and repeatability was close to the repeatability of the first two trials (0.39 versus 0.50). This suggests that rank disturbance was the prime cause for the decrease and loss of repeatability. However, the decrease of variance due to habituation should not be ignored either; exposing sharks more frequently could further investigate this effect. Regardless of the causes, if one is interested in testing reaction before habituation such as reaction to novelty, our results illustrate the need to acknowledge rank disturbance and general effect of habituation, either using random mixed models if data resolution allows or by limiting the number of trials in the analyses.

Personality-related plasticity differences have been demonstrated in several taxa (Mathot et al., 2012). Interestingly, we found strong differences in the direction of change, with some individuals decreasing, some not changing and others increasing their ROM. Similarly, strong differences between individuals in their habituation/sensitization rate (to a low-risk predator) and a negative correlation between exploration score and these differences were found in Iberian wall lizards, *Podarcis hispanicus* (Rodríguez-Prieto et al., 2010). Furthermore, different inbred strains of mice, *Mus musculus*, varied in their direction of activity change (decrease, increase or no change) with trial repetition (Bolivar, Caldarone, Reilly, & Flaherty, 2000) suggesting a genetic effect on the expression of exploration and habituation/sensitization. These studies support our finding but it has to be noted that the semiwild conditions of our experimental set-up prevented total experimental control.

An interesting avenue for future investigations is to study the proximate and ultimate causes of this personality-related difference in plasticity. Discussing our results in such a context is premature but empirical and theoretical investigations help direct future research. In the particular case of exploration and habituation/sensitization, insights can be gained from studies on rodents. In these animals, there is evidence for neurochemical, morphological and genetic factors underlying habituation (Leussis & Bolivar, 2006). These studies do not always focus on individual differences but might help explain the proximate causes of our observed effects. For instance, glucose is known to impact habituation in rodents (Leussis & Bolivar, 2006) and, likewise, individual differences have been found in juvenile lemon shark blood glucose during stressful events (Brooks et al., 2011). It would thus be informative to correlate these blood parameters with behaviour in a novel open field and habituation/sensitization rate. Another explanation for the observed variation could be cognitive differences between individuals (Carere & Locurto, 2011; Guillette, Reddon, Hurd, & Sturdy, 2009; Sneddon, 2003). These hypotheses could be further investigated as classical conditioning experiments

in this species suggest interindividual cognitive differences (Gruber & Schneiderman, 1975). Overall, little is known about how such personality-related differences in plasticity (including habituation/sensitization) can be selected and under which environmental conditions. Such causes have, nevertheless, recently been discussed (Dingemans & Wolf, 2013; Mathot et al., 2012) and their demonstration needs a clear and broad understanding of the study species and study system.

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