

**The Effects of Sex, Physical Traits, and Parasites on Painted Turtle
(*Chrysemys picta*) Behaviour**

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Abstract

While the study of animal personality is gaining in popularity, no adequate tests had been proposed to investigate it in turtles prior to my field work. In this study, I designed two new tests to assess turtle aggressive and boldness behaviours. Additionally, I tested the hypotheses that the sex and physical traits of an individual are linked to specific behaviours, and that personality traits created variation in parasite load in painted turtles (*Chrysemys picta*). I predicted that males and females would differ in terms of personality traits, as well as bigger and smaller individuals. I also predicted that bolder individuals would have more leeches than shyer individuals, as bolder individuals might use bigger areas and be more exposed to parasites. The results of my behavioural tests showed that less aggressive individuals seemed to be shyer. However, I found no other significant correlations between the sex, physical traits, number of leeches, and behavioural measurements. My findings are a good starting point for future studies to extend knowledge on turtle personality.

Keywords : Behaviour, Personality, Physical traits, Sex, Ectoparasites, Turtle

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Introduction

Animal 'personality' is consistent and repeatable inter-individual differences in behaviour through time and/or across contexts (Dall et al. 2004, Réale et al. 2007). Personality implies that individual behaviours are well suited in some situations, while being inappropriate in others, hence the observable between-individual variation in behaviour in a variable environment. For instance, an aggressive behaviour could be helpful when competing for food, but could be detrimental in presence of a predator (Sih et al. 2004). Sih, Bell, and Ziemba (2004) also proposed that some types of behaviour co-evolved because certain combinations of traits (e.g., exploratory and aggressive) are advantageous compared to others (e.g., exploratory and submissive). Those correlated types of behaviour are known as behavioral syndromes.

It is argued that the sex of an animal may select for favour a particular behavioral syndrome. Indeed, as males and females are subjected to different selective pressures, animals may exhibit sex-specific behaviours (Schuett et al. 2010). One suggested mechanism is that sexual selection can induce mate preferences, as females could prefer males with behavioural traits that imply better 'quality' (e.g., genetic quality), or compatible males (assortative and disassortative mating). There is evidence of sex-specific behaviours in various taxa (Whoriskey 1991, Holder et al. 1991, Johnston and File 1991, Lonstein and De Vries 2000, Aguilar et al. 2003, Bales and Carter 2003, Harris et al. 2010, Manson and Perry 2013, Rokka et al. 2014, van Overveld et al. 2014, Fresneau et al. 2014, Patrick and Weimerskirch 2014, Timm et al. 2015, Han et al. 2015). For example, Harris et al. (2010) found that male guppies were bolder than females, and that the behaviour was

strongly shaped by predation pressure. Indeed, females are more cautious than males because they are limited in their reproductive success by their survival and longevity. Finding mates is not as important for females as it is for males, because female guppies are able to store sperm. Conversely, males are less cautious as they can maximise their fitness by finding more mating opportunities, at the cost of higher risk of predation. Therefore, the sex of an individual can shape its personality traits.

Additionally, phenotypic traits can explain part of the behavioural variation, because some behavioural traits may only function well with some physical attributes, which are, sometimes, only possessed by one sex. Many studies provide evidence of a relationship between body size and personality (Biro and Stamps 2008, Seda et al. 2012, Funghi et al. 2015, Maillet et al. 2015, De Winter et al. 2016, Mayer et al. 2016, Kelleher et al. 2017, Anderson Berdal et al. 2018), which is often linked to sex polymorphism and sex-specific behaviour. Maillet et al. (2015) observed that longer female garter snakes explored less than smaller ones and that longer males explored more than smaller ones. However, not all studies found evidence of body size influence on personality (Carter et al. 2012). De Winter et al. (2016) suggested that the absence of correlation can be explained by ecological factors, such as predation. For instance, smaller fish might be more exploratory than bigger ones, as bigger conspecifics are preferred by a predator. However, the behaviour would not be universal as the predation context can vary in time and space. If the predator did not select for a specific size of prey, smaller and bigger fish could show equally bold behaviours.

To identify other evolutionary mechanisms that could explain inter-individual differences in behaviour, recent studies have investigated the potential relationship between parasites and personality traits in many taxa (Barber and Dingemanse 2010; Kortet, Hedrick, and Vainikka 2010; Ezenwa et al. 2016; Seaman and Briffa 2015; Barber et al. 2017; Koprivnikar, Gibson, and Redfern 2012; Dunn, Cole, and Quinn 2011; Patterson and Schulte-Hostedde 2011; Bohn et al. 2017; Zohdy et al. 2017; Horváth et al. 2016; Sih et al. 2018). Barber and Dingemanse (2010) propose that the relationship may be due to the susceptibility of some personalities, which are more likely to acquire parasites (e.g., more exploratory individuals). Boldness seems to be one of those traits as bolder male chipmunks have higher parasite loads (Patterson and Schulte-Hostedde 2011). Sih et al. (2018) explain that parasite transmission might depend on personality in a study on lizards, because personality traits affect space use, which in turn affects transmission networks and parasite loads. Alternatively, Barber and Dingemanse (2010) also suggest that parasite infection might cause a change in the host behaviour, as parasites have an effect on the host. For instance, antipredator behaviour in prey can be modified by the presence of trophically transmitted parasites to make them more vulnerable to predation and ease parasite transmission to potential predators (Lafferty and Morris 1996). As parasites live to the detriment of their host, they can have a colossal impact on fitness and survival of individuals (see among others Gibbons et al. 2000). Thus, the study of parasite and personality is of major interest.

Even though the study of personality is gaining in popularity and its implications for ecology and evolution are undeniable (Dall et al. 2012, Wolf and Weissing 2012), very few studies have linked personality to morphological factors or parasitism in turtles. Yet, many

turtle species are endangered, and a better understanding of personality traits could be crucial in conservation strategies, such as captive breeding and recovery programs (Smith and Blumstein 2008, Merrick and Koprowski 2017). For instance, in the context of anthropogenic disturbance, disturbance-tolerant individuals (such as bold, active or explorative ones) are expected to use crossing structures more or to enter traps more (see among others Lowry, Lill, and Wong 2013; Atwell et al. 2012). To address the gaps in literature, I wish to test the hypothesis that variation in parasite load between individuals is caused by their personality traits, because some personality traits (e.g., boldness) should promote parasite transmission more than others (e.g., shyness). For instance, more active or exploratory individuals use larger habitat areas, which could expose them to more parasites (Barber and Dingemanse 2010). I predict that bolder and more aggressive individuals should have more leeches than shyer individuals. Furthermore, as some behavioural traits may only function well with a given physical trait, which is sometimes only possessed by one sex, I hypothesize that the sex and physical traits of an individual are linked to specific personality traits. However, as the relationship between those variables seems to be determined by various ecological factors and the studied species, I cannot make any precise predictions. Instead, I propose that, generally, females and males will differ in terms of behavioural traits, as will smaller and bigger individuals. My hypotheses will be tested on Painted turtles (*Chrysemys picta*).

In addition, I intend to explore new ways to measure behaviour. Researchers have previously attempted to test turtle behaviour — or fitness, phenotypic quality, and performance — by measuring the righting response (Steyermark and Spotila 2001, Freedberg et al. 2004, Delmas et al. 2007, Micheli-Campbell et al. 2011, Ibáñez et al. 2013,

Sim et al. 2015, Carter et al. 2016, Chiari et al. 2017, Polich et al. 2018). However, Davy, Paterson, and Leifso (2014) dismissed that method as it did not pass the test of rank repeatability. The righting time differs greatly among trials and did not prove to always rank individuals in a consistent order. Other analogous studies measured reflexes in turtles such as response to startles, tactile stimuli (LeDain et al. 2013, Stoot et al. 2013, Gutowsky et al. 2017), and the degree of activity (Cairns et al. 2017) to detect behavioural impairments in the context of bycatch in fishing nets. Although those tests are well adapted to their context, they do not allow to test personality. Since the righting response has failed to be an accurate way of measuring behaviour, and since no other test specifically apply to turtle personality, there is a glaring need for new approaches. In this study, I propose two new tests to assess turtle behaviour.

Methods

Study Animals, Study Sites, and Morphological Measurements

The experiment was carried out from early July to mid-August 2018 in the Rideau canal waterway (Ontario, Canada). The sites were in different lakes (Figure 1) based on the presence of suitable basking sites, such as stumps (Peterman and Ryan 2009).

The Painted Turtle (*Chrysemys picta*) is widely found across North America, and is easily recognized by its bright red and yellow colours on the skin and the plastron. Turtles were mainly captured with unbaited hoop nets, but also occasionally with long-handled dip nets or by hand. All hoop nets were checked every 24 hours or less, and captured individuals

were carried in the boat for several measurements to be taken. All individuals were released immediately after all tests and measurements were done at the exact location of capture.

Sexual dimorphism was used for sexual identification, based on foreclaw length, tail length, and general dimension (Ernst and Lovich 2009). To measure the variation in physical traits between individuals, carapace measurements, tail length, and mass were recorded. Four morphological measurements were taken with a vernier caliper ($\pm 0,05$ cm): maximum carapace length (CL), maximum plastron length (PL), maximum carapace width (CW), and maximum carapace height (CH). Tail length (TL) was measured with a plastic ruler ($\pm 0,05$ cm). It was chosen as one of the variable of interest because males have longer tails. Thus, this variable can account for both sex and physical trait variation. Turtles were weighed with a spring scale (± 5 g). As a measure of parasite load, we noted the number of leeches on each individual. Turtles were given a unique code on the marginal scutes with a triangular file for future identification (Nagle et al. 2017).

Measuring Behaviour

Two tests were done with each individual to assess their behaviour. Tests were all done during the daytime. Before beginning our observations, we divided their aggressive behaviour in three operational categories based on previous studies (Bury and Wolfheim 1973, Bury et al. 1979, Lovich 1988, Ernst and Lovich 2009) and our own observations:

0 - Avoidance. The head and appendages are retracted in the shell.

1 - Curiosity and escape attempts. The turtle's head is protracted, it is looking around, and/or it is pushing with its legs in an attempt to free itself.

2 - Gaping, open-mouth gesture, and/or biting. Gaping is an opening of the mouth not directed towards others, as opposed to the open-mouth gesture which is directed towards others to display the bright colors of its interior and scare them. Biting is observed when the turtle clamps its mouth onto something.

Although the choice of classifying some behaviours together or apart was arbitrary, these are the only categories that were easily distinguished in the field. Their behaviour was observed while taking carapace measurements, and turtles were scored with the highest number (maximal aggression score) that would represent their actions during that short period of time (about 1 minute). For instance, if an individual tried to escape and tried to bite, it would be scored as a 2. To ensure a standardized procedure, the measurements were the first manipulation to be made after the animals were taken out of the hoop net, they were always taken in the same order (CL, PL, CW, CH), and they were done as quickly as possible.

For the second experiment, we recorded the escape time from a floating platform to the water. To do so, we installed a 23 cm x 28 cm x 5,5 cm orange styrofoam board (Figure 2) close to the capture location, and attached it to the boat with a rope at a maximum distance of 150 cm from the boat. Based on Kashon and Carlson (2018), each turtle was first put in a dark closed container for 3 minutes at the end of the other manipulations as an accustomization period, to ensure that they all had the same treatment before the last test. The turtle was then quickly placed in the middle of the platform, parallel to the boat in a way that it could see the observer, and we started the chronometer. The chronometer was stopped once the turtle entered the water.

To control for the strong influence of temperature on performance in ectotherms (Huey and Stevenson 1979, Kingsolver and Huey 2008), we used both maximum and mean daily temperatures, recorded at the closest weather station of Environment Canada, in Kemptville, Ontario.

Statistical Analyses

I used a linear mixed effects model to identify if sex, parasite load and body size were related to the escape time from the platform, using the *lme4* package (Bates et al. 2016). I started with a correlation matrix to visualize the correlation coefficients between each pair of predictors. Then, to avoid collinearity between my variables, I proceeded with a variance inflation factors (VIF) analysis. I removed one by one the variables that had the highest scores until all remaining variables had values below 3 (Zuur et al. 2010). I applied a logarithmic transformation to the escape time data to ensure that the conditions of application were respected, and standardized my variables (mean of $x = 0$; SE of $y = 0.24$). The identity of the lakes and the sites were included as random variables and were kept in the model given that they were significant. I proceeded by backward elimination and did an analysis of variance (ANOVA) to examine if the removal of each variable was significant. Moreover, I tested the escape time repeatability with the *rptR* package (Stoffel et al. 2017) and a *Poisson* distribution. Finally, I tested the relationship between aggressive behaviour and escape time from the platform by comparing the time spent on the platform for each category with an ANOVA. All the analyses were performed in R version 3.5.3 (R Core Team 2019).

Results

The correlation matrix showed high correlations between some pairs of predictors (Figure 3), which was corrected subsequently by removing some variables from the model following the VIF analysis (see Table 1 for the list of all initial variables). No significant effects were found between the resulting variables and escape time from the platform (Table 2 & Figure 4). Escape time repeatability was 0.268 ± 0.045 ($N = 5$). I found that individuals that were given the lowest aggression score (0) stayed significantly longer on the platform compared to individuals with a score of 1 or 2 (Figure 5).

Discussion

I hypothesized that the variation in parasite load was caused by personality traits and predicted that bolder and more aggressive individuals would have more leeches than others. I also hypothesized that the sex and physical traits, such as body size, are linked to specific personality traits. I predicted that males and females would show differences in the time spent on the platform, as well as smaller and bigger individuals. Although I did not find that sex, physical traits or parasite load were related to aggressive or boldness behaviours, I found that more aggressive individuals were spending less time on the platform.

Boldness and aggressive behaviours

Individuals with the lowest aggression score (0), which were retracted in their shell all along the manipulations, were staying significantly longer on the platform than other individuals, suggesting that they could be shyer than those with a higher aggression score

(1 and 2). Pich et al. (2019) found similar results on the eastern box turtles (*Terrapene carolina*), as individuals performing fewer defensive behaviours (e.g., fleeing, biting) stayed hidden longer in their shell in a simulated predator context. This relationship could be due to the higher predation risk experienced by bolder individuals, who have a propensity to take risks and might have a higher encounter rate with a predator (Hulthén et al. 2017). Indeed, the proactive defensive strategies could play a crucial role in reducing the predation cost of bolder behaviours, while hiding in the shell could be sufficient for more reactive individuals (Pascual and Senar 2014). My results suggest that a behavioural syndrome might exist, as more aggressive individuals are also bolder, but more behaviours would have to be tested in order to infer a syndrome. Also, to confirm that the escape time from the platform really is a measure of boldness, the test could be improved by adding a component in which latency to emerge from the shell is measured. The time spent in a refuge is known to be an indicator of shyness (Ibáñez et al. 2013).

I obtained a repeatability of 0.27 for the escape time from the platform, which corresponds to what is found in other studies (mean = 0.37; Bell et al. 2009). It is consistent with other findings that suggest that boldness is repeatable and even heritable (van Oers et al. 2004). However, given the limited sample size of recaptured individuals (N = 5), I consider that the result is not conclusive. In addition of the lack of power it causes, it is possible that my sample is not representative of the population. Therefore, my findings should be considered with caution. Future studies should test more extensively those new behavioural tests to confirm their repeatability, as they could be predictive of the performance in other tests. Indeed, Pich et al. (2019) found that bolder individuals were consistently bolder across contexts, as they exhibited bolder behaviours than conspecifics when handled by humans

and in the context of a predator attack (Pich et al. 2019). Thus, performance in one test (e.g., escape time from a platform) could be predictive of performance in another (e.g., predator attack).

During the entire period of the test, turtles could see and hear the experimenters in the boat, which might have influenced their behavioural response. This could be problematic as the intensity of the noise and the distance between the platform and the observers fluctuated between assays, and even during a trial, resulting in a lack of standardization. A greater and fixed distance between the observers and the animals, and a reduction of the talking between researchers would be imperative to minimize the effect of experimental conditions. Furthermore, to maximise standardization, the tests should also be done in controlled laboratory conditions. It would allow us to investigate the behaviour across contexts and test whether a within-individual consistency exists. Moreover, future studies should evaluate the effect of both novelty and habituation to understand if and how the perception of risk is altered and how it modifies boldness behaviour.

Sex and physical traits

There was no difference between males and females, or bigger and smaller individuals in terms of time spent on the platform. My results are consistent with a recent study on eastern box turtles (*Terrapene carolina*) that also found no effect of body size and sex on boldness behaviour (Kashon and Carlson 2018). This suggests that painted turtle behaviour is shaped by other factors. For instance, it would be interesting to investigate the role of metabolic rates (Niemelä and Dingemanse 2018) and thermoregulation (Michelangeli et al. 2018) in personality, as the need to thermoregulate is fundamental in ectotherms. Indeed, some

individuals might perform better at higher body temperatures, resulting in higher activity levels – e.g. more active and exploratory behaviours, and higher metabolic rates.

Parasites

There was no relationship between behaviour and parasite load between individuals. I predicted that the number of leeches would be higher on bolder individuals, as their behaviour would promote parasite transmission. However, my prediction was not supported by my data, as individuals staying only a few seconds on the platform (i.e., bolder individuals) and individuals staying longer (i.e., shyer individuals) had indistinguishable parasite loads. It would be an interesting avenue to integrate movement, space use, parasite load and personality in future research on aquatic reptiles, as did Sih et al. (2018) with a terrestrial lizard, because it offers a more complete understanding of the mechanisms underlying parasite transmission.

Conclusions

This study aimed to fill the gaps in literature by proposing new tests to measure turtle behaviour, which can be easily done in the field. Although the tests are promising, they need to be further evaluated to confirm their reliability. Both of my hypotheses were not supported by my data as I did not find any effects of sex and physical traits on behaviour, and I did not find that bolder individuals had bigger parasite loads. Future studies should continue to explore what variables shape behaviour and what ecological consequences they have, putting the emphasis on thermoregulation and space use.

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Table 1. Mean, standard deviation, and minimum and maximum values of all measured variables.

Variables	Mean	Standard deviation	Minimum	Maximum
Carapace length (mm)	141.1	16.88	70.0	183.0
Plastron length (mm)	129.6	16.11	62.0	169.0
Carapace width (mm)	104.4	11.19	61.0	133.0
Carapace height (mm)	49.29	6.95	28.0	69.0
Tail length (mm)	54.98	7.55	25.0	69.0
Mass (g)	359	117.35	60	720
Number of leeches	0.91	1.69	0	11
Mean temperature (°C)	20.8	2.43	17.6	27.7
Maximum temperature (°C)	28.5	2.18	24.5	34.4
Escape time (s)	68.2	148.32	0.3	900.0

Table 2. Full model of the effect of explanatory variables on the escape time. I used a linear mixed-effect model with standardized data, previously selected by a variance inflation factors analysis, and controlling for the different lakes and sites. Intercept values were obtained from the null model.

Variables	Estimate	Standard Error	t value	p value
Intercept	2.0734940	0.4637739	4.4709150	< 0,01
Tail length	-0.0094334	0.2948580	-0.0329940	0.97
Presence of leeches	0.0307412	0.5782794	0.0531595	0.96
Maximum temperature	-0.1326183	0.4238189	-0.3129127	0.75
Number of leeches	-0.1327642	0.2183401	-0.6080613	0.54
Carapace width	-0.1648414	0.2502823	-0.6586220	0.51
Sex (males)	-0.4030812	0.4669833	-0.8631598	0.39
Mean temperature	0.5989624	0.3231062	1.8537640	0.06

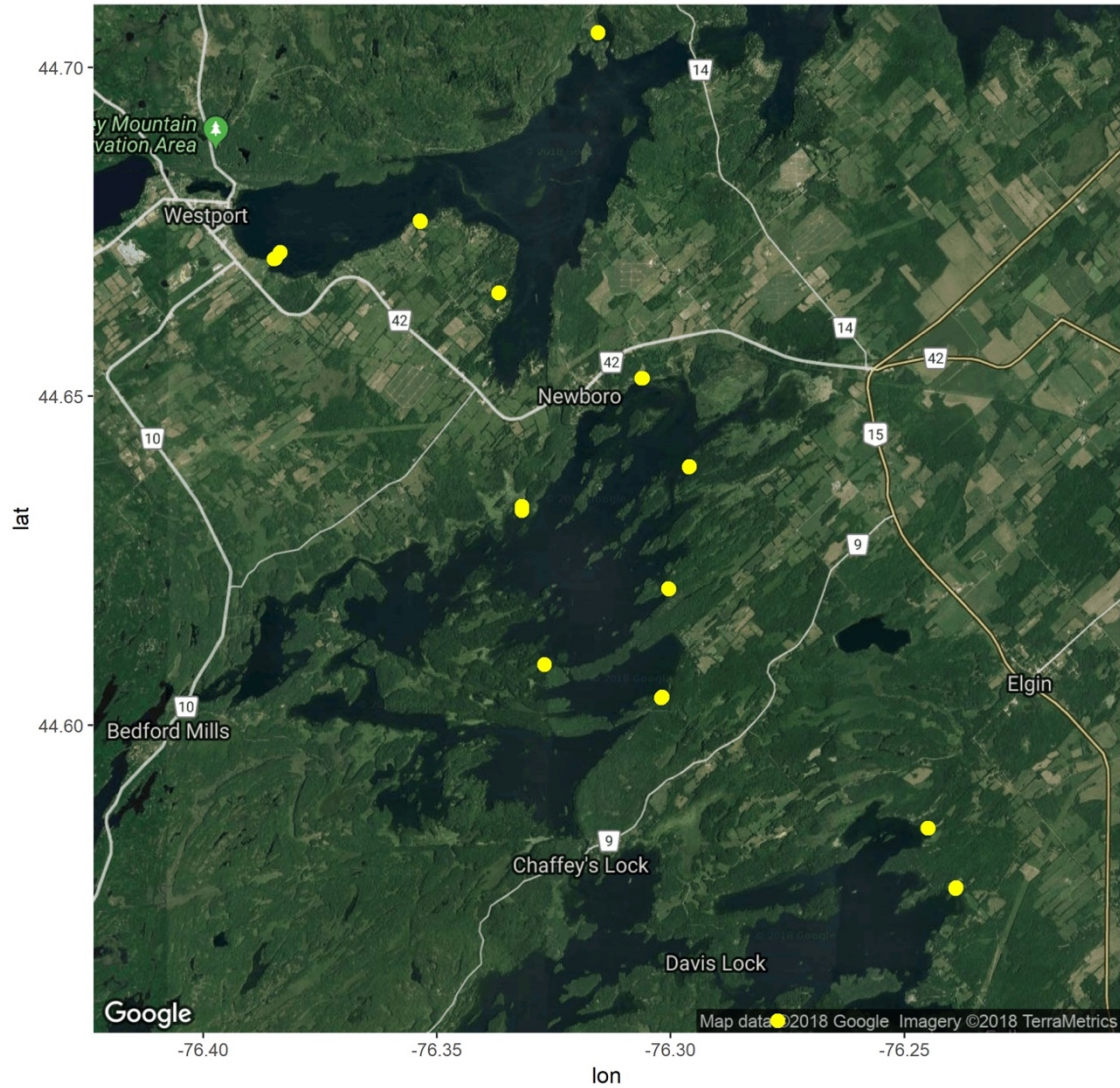


Figure 1. Map of the study sites (yellow dots), near Queen's University Biological Station. The sites are located in different lakes (Sand, Clear, Newboro, and Upper Rideau) in the Rideau canal waterway.



Figure 2. Measuring the escape time from a styrofoam platform to water. The Painted turtle is retracted in its carapace.

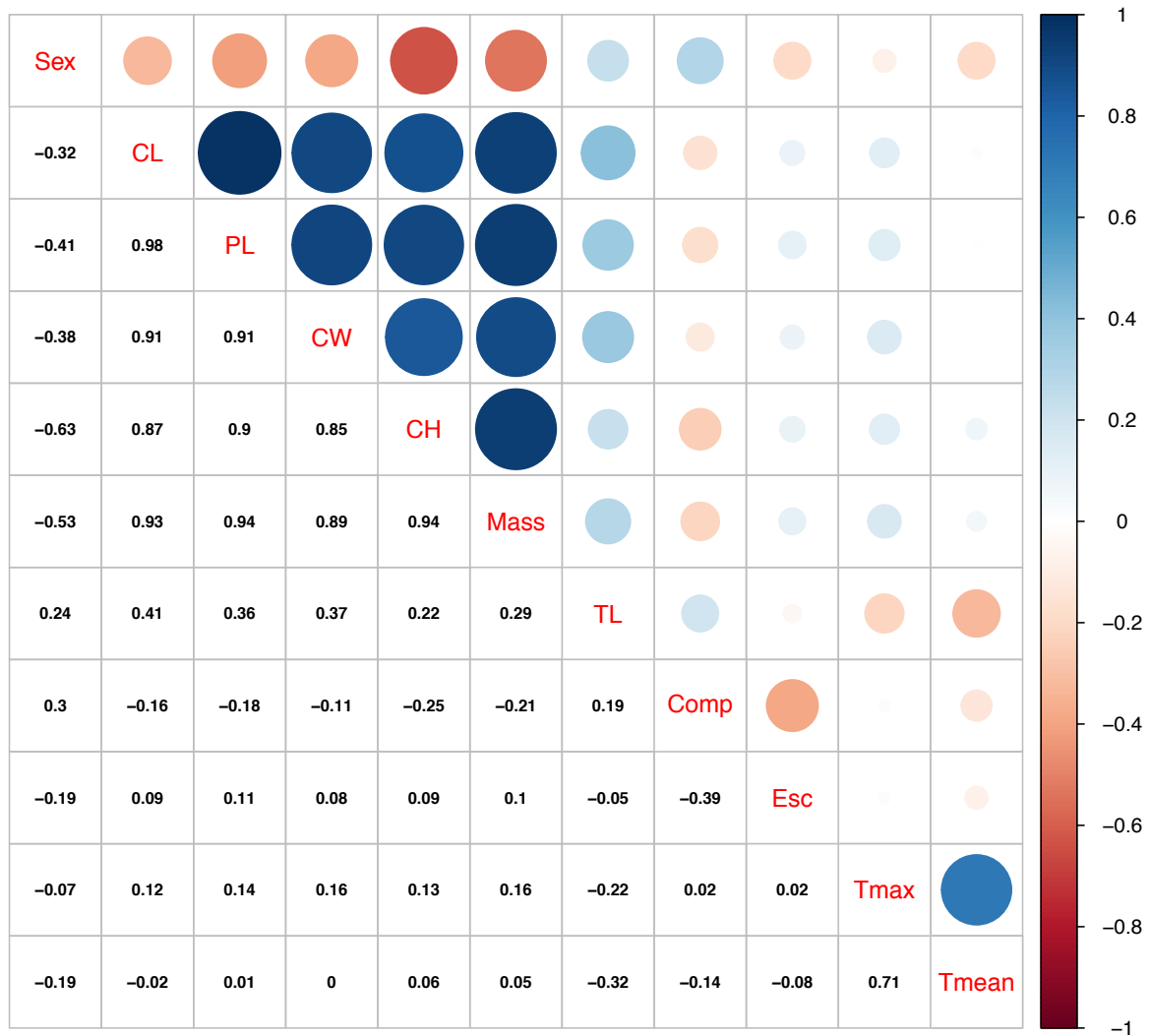


Figure 3. Correlation matrix of all measured variables. This correlogram displays bigger circles when the correlation coefficient is higher. The color blue means that the correlation is positive, while the color red means it is negative. Legend: CL = Carapace length; PL = Plastron length; CW = Carapace width; CH = Carapace height; TL = Tail length; Comp = Maximal aggression score (0-2); Esc = Escape time from the platform; Tmax = Maximal daily temperature; Tmean = Mean daily temperature.

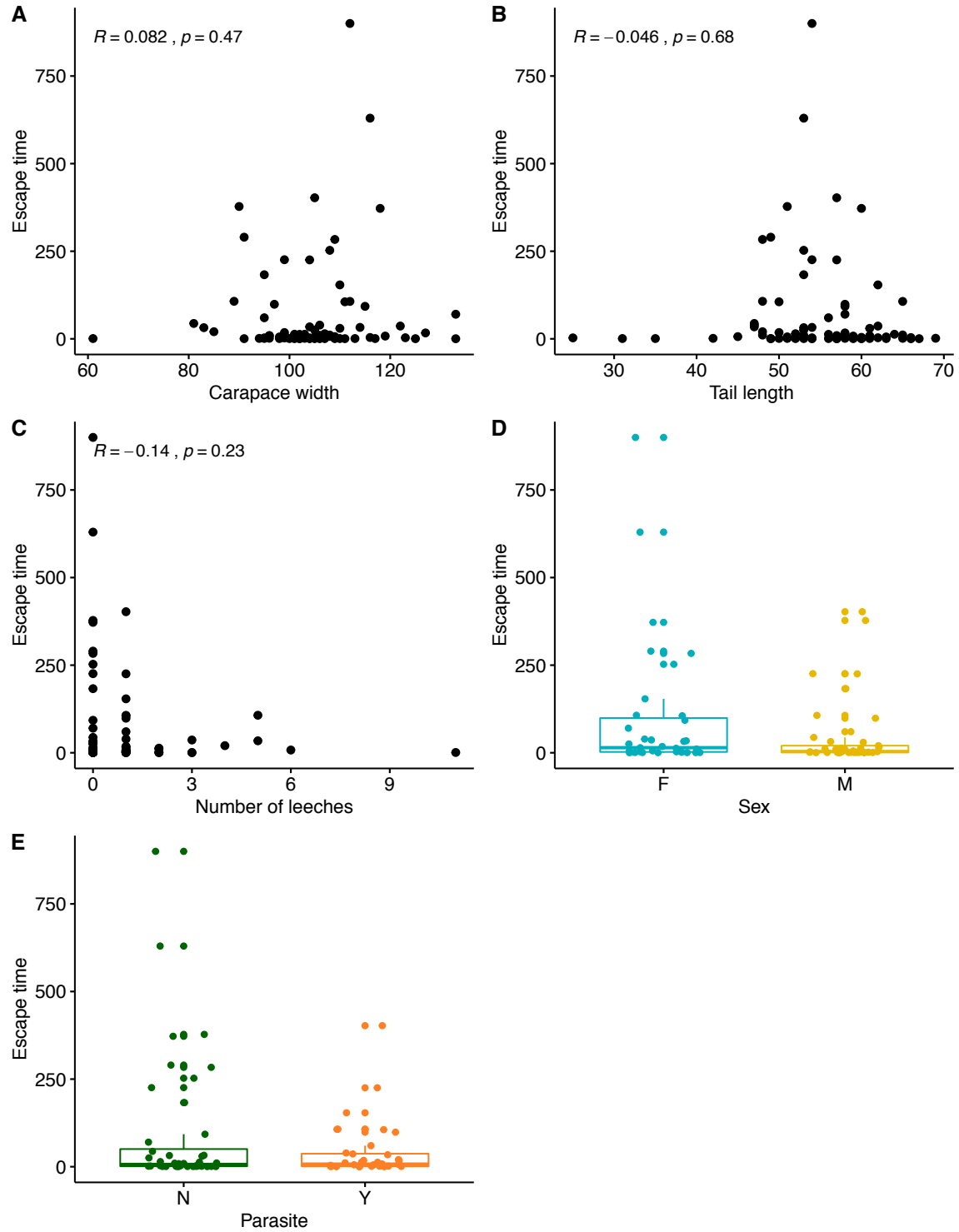


Figure 4. Relationship between the escape time (s) and the A) carapace width (mm), B) tail length (mm), C) number of leeches, D) sex (blue = female, yellow = male), E) presence of leeches (green = no leeches, orange = presence of leeches). Each point represents an individual (N=90). In the boxplots, the central line is the median. No significant correlation were found.

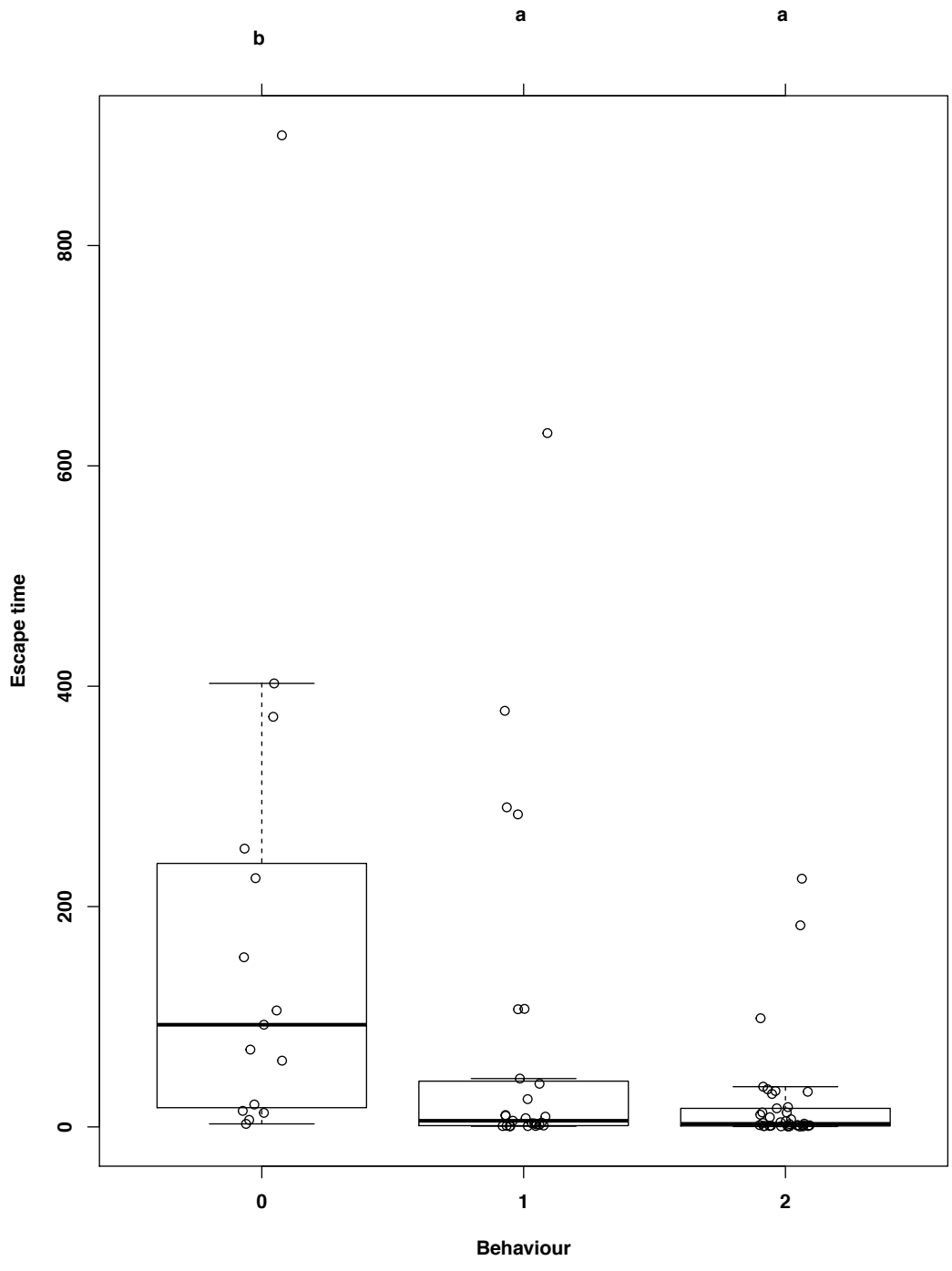


Figure 5. Relationship between the escape time (s) and operational categories of behavior. Each point represents an individual (N = 90), the central line is the median, and the whiskers are the minimum and the maximum. The compact letter display at the top of the graph shows that the category 0 significantly differs from 1 and 2.