LUANA DIAS LEITE CARDOSO

ORDER AND DISORDER ARISING FROM ALARM: HOW DO TERMITES BEHAVE FACING POTENTIAL THREATS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

Orientador: Og Francisco Fonseca de Souza

Coorientadora: Gladys Julieth C. Quiroga

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

C268o
2021

Cardoso, Luana Dias Leite, 1993-
Order and disorder arising from alarm : how do termites behave facing potential threats / Luana Dias Leite Cardoso. – Viçosa, MG, 2021.
45 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Inclui apêndice.
Orientador: Og Francisco Fonseca de Souza.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f. 28-30.

1. Térmita - Comportamento. 2. Reação de alarme. I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22. ed. 595.736

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB6 2523

LUANA DIAS LEITE CARDOSO

ORDER AND DISORDER ARISING FROM ALARM: HOW DO TERMITES BEHAVE FACING POTENTIAL THREATS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

APROVADA: 30 de julho de 2021.

Assentimento:

Luana Dias Leite Cardoso

Luana Dias Leite Cardoso
Autora

Og Francisco Fonseca de Souza
Orientador

Aos que me acompanham e vibram comigo as nossas conquistas.

Acknowledgments

Agradeço primeiramente a minha mãe, avó e demais familiares pelo apoio e por sempre acreditarem em mim.
Agradeço ao Og por todos os ensinamentos e pela paciência.
Agradeço à Julieth pelos ensinamentos, pela paciência, pelo apoio e pela grande ajuda nesse caminho.
Agradeço à Rebeca Rosengaus pelos ensinamentos e trocas de experiências.
Agradeço aos atuais e ex Termitóides Marco, Reinaldo, Raphael, Zé pelo companheirismo e em especial Elisa, Dina, Lara e Lívia por toda ajuda científica e emocional.
Agradeço aos colegas da pós graduação, em especial a Kárenn que se tornou uma grande amiga.
Agradeço aos amigos da vida e em especial Elisa, Rosana, Renan, Caio, Snaydia, Leles e Anna.
Agradeço à Universidade Federal de Viçosa e ao Departamento de Pós-Graduação em Entomologia pela oportunidade e em especial à Eliane que sempre esteve pronta a ajudar.
Agradeço ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de estudos.
O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

"Traveler, there is no path. Paths are made by walking."

Antonio Machado

Abstract

CARDOSO, Luana, M.Sc., Universidade Federal de Viçosa, July, 2021. **Order and disorder arising from alarm: How do termites behave facing potential threats**. Advisor: Og Francisco Fonseca de Souza. Co-advisor: Gladys Julieth Castiblanco Quiroga.

Individuals respond differently to distinct intensities of an alarm signal and that, ultimately, could affect group order. Under signals of low intensity, a few individuals are bound to be affected and to respond while the majority would remain doing regular activities. At this point, a high variety of behaviours are observed so that the group is perceived as disordered. As alarm intensity gets higher, a low variety of behaviours and group cohesion may happen, since most individuals would perceive the signal and respond similarly. That in fact is what we aim to show in this work. Here we test the hypothesis that under alarm the order of social groups at individual scale is modulated by the intensity of the signal. Our results support that, under low intensity of the signal, individuals tend to behave uncoordinatedly. Under higher intensity of the signal, the opposite is observed. This happens because under low doses of the stimulus, the diversity of behaviours is increased whereas under high doses the diversity of behaviours decreased as predicted by our hypothesis. Ultimately, a group exhibiting low variety of behaviours correspond to a more cohesive group, or a group exhibiting collective behaviour. It remains to be tested whether even more intense alarm signals, such as risk of predation, would compromise this cohesion, by returning individuals to their idiosyncratic behaviours.

Keywords: Order and disorder. Termites. Alarm behaviour.

Resumo

CARDOSO, Luana, M.Sc., Universidade Federal de Viçosa, julho de 2021. **Ordem e desordem emergindo do alarme: como os cupins se comportam diante de uma potencial ameaça**. Orientador: Og Francisco Fonseca de Souza. Coorientadora: Gladys Julieth Castiblanco Quiroga.

Indivíduos respondem de diferentes formas a distintas intensidades de um sinal de alarme e isso pode afetar organização de grupo. Sob sinais de baixa intensidade, poucos indivíduos são afetados e respondem, enquanto a maioria continua fazendo atividades regulares. Nesse ponto uma alta diversidade de comportamentos é esperada e o grupo é percebido como desorganizado. Quando o sinal de alarme se torna mais intenso, uma baixa diversidade de comportamentos e coesão de grupo podem ocorrer, visto que muitos indivíduos percebem o sinal e respondem a ele de forma similar. Aqui, testamos a hipótese de que sob alarme, organização da resposta em animais sociais a nível de indivíduo é moldada pela intensidade do sinal. Nossos resultados mostram que sob sinais de alarme de baixa intensidade, indivíduos tendem a se comportar de forma desordenada. Enquanto sob altas intensidades do sinal de alarme o oposto é observado. Isso ocorre, pois sob sinais de baixa intensidade, a diversidade de comportamentos aumenta, enquanto sob sinais de alarme de alta intensidade, a diversidade de comportamentos diminui, como previmos em nossa hipótese. Em última análise, um grupo exibindo baixa variedade de comportamentos corresponde a um grupo mais coeso ou a um grupo exercendo comportamento coletivo. Ainda é necessario testar se sinais de alarme ainda mais intensos, como um risco de predação, comprometeriam essa coesão por retornar os indivíduos a seus comportamentos idiossincráticos.

Palavras-chave: Ordem e desordem. Cupins. Comportamento de alarme.

List of Figures

List of Tables

Contents

Chapter 1

Introduction

In a society, individuals are stimulated by internal and external conditions, and that affects social order. Internal conditions are intrinsic of a given society, such as social hierarchy or the imposed social rules in human societies. External conditions are extrinsic from the society, coming from environmental phenomena or any unexpected event. On alarm signal, for example, conditions changes and disorder is frequently expected. However, disorder is not the sole result af alarm. In this paper we aim to demonstrate that individuals tend to show both disorder and order in different intensities of alarm.

Alarm is defined as a warning of a possible danger (Maschwitz, 1967), such as the appearing of a predator, a competitor, a pathogen or anything potentialy harmful. Alarm induced behavioural responses have been shown in animals ranging from invertebrates (Jacobsen and Stabell, 1999) to vertebrates (Speedie and Gerlai, 2008). In animals as well as in humans, alarm intensity provokes changes in the diversity or intensity of its matching behavioural responses (Bliss et al., 1995). Diversity of behaviours can be related to systems order and information variety (Mikhailovsky and Levich, 2015). When there is a high diversity of behaviours, there is a high variety of information and the system may appear disordered (Mikhailovsky and Levich, 2015). On the other hand, when there is a low diversity of behaviours, there is a low variety of information and the system may appear ordered (Mikhailovsky and Levich, 2015).

Lets take us humans as an example. Observing humans in a closed environment, such as a night club, a high diversity of behaviours are observed. Some people dancing, others drinking, others flerting or talking and so on. When a low intensity alarm occurs, an altercation at the bar for example, the individuals near to the quarrel area will be affected. It is possible that some people inform the security, some other try to separate the opponents, but most people will remain doing what they were doing. Thus, because "new" behaviours have been added to the group, an increase in the variety of behaviours and a consequent increment in disorder is observed.

On the other hand, if a dangerous situation occurs, a fight for example, many individuals could perceive the alarm, act upon it, stopping what they were doing before. They could, for instance, try to leave the place being copied by others and that would create herd effect (Kelley et al., 1965). Because the previous diversity of behaviours were replaced by a limited set of actions(i.e. leave the room) we would now observe a change from disorder to order.

However, if the alarm stimulus is highly dangerous, a fire for example, panic or high alarm response is triggered. In that case, we may observe an increase of other behaviours, such as running and pushing (Helbing et al., 2002), generating system's disorder.

Analysing the behaviour of social insects it is possible to observe certain regular activities. Bees peform the waggle dance to indicate the location of the food source (Grüter and Farina, 2009), termites use substrate-borne vibrations to signal quality of a food resource (Evans et al., 2007) and experienced ants teach naive ants how to go from nest to food source (Franks and Richardson, 2006). While the above behaviours may find a parallel in humans, it is under danger that social insect exhibit stronger similarity to humans.

Ants running for survival, for instance, may not succeed escape in the same way as crowding humans (Altshuler et al., 2005), because panicked ants block the interceptions of escaping routes (Dias et al., 2012).

By contrast, termites seem to perform a highly ordered escaping behaviour at the group scale (Wang et al., 2016). However, it remains to be unveilled if the order at the group scale percolates to the individual scale. If so, we would profit a lot knowing the factors that modulate such an order.

So, in this work we aim to test the hypothesis that in danger situations the order at individual scale of social groups is modulated by the intensity of the alarm signal. To do so, we exposed 900 *Constrictotermes cyphergaster* termites from fifteen colonies to different alarm intensities and analysed the diversity of behaviours performed by them under different intensities of alarm. We show that under low alarm *Constrictotermes cyphergaster* termites increase their diversity of behaviours, whereas under high alarm intensity, the opposite is observed. Futhermore, here we argue that the order at individual scale in alarm context depends on the intensity of the danger signal.

Chapter 2

Materials and methods

2.1 Rationale

Here we tested if the intensity of alarm modulates the order at individual scale. To do so, we video-recorded termites being exposed to varying types and intensities of stimuli, to simulate increments in alarm intensity. Four types of alarm were used, physical, physicochemical, chemical and biochemical. Different doses of chemical and biochemical stimuli were applied. We analysed the behavioural responses of the termites and constructed an ethogram to compare the diversity of behaviours performed by individuals before and after stimulus insertion.

For better understanding, here we define a few terms.

1. Order: decrease of behaviours diversity
2. Disorder: increase of behaviours diversity
3. Before insertion: the moment before the insertion of stimulus
4. After insertion: the moment during and after the insertion of stimulus

2.2 Termites collection

Fifteen colonies of *Constrictotermes cyphergaster* (Silvestri, 1901) were collected in April of 2017 in the municipality of Sete Lagoas (27°19'S, 14°44'W), Minas Gerais, Brazil (for more information see (Nunes et al., 2019)). From each one of these colonies 60 termites (12 soldiers and 48 workers) were extracted and distributed in four groups of 15 termites (3 soldiers and 12 workers) each.

2.3 Experimental setup

Each group of termites was placed in an arena composed by a flexible floor and a plastic Petri-dish (diameter = 53 mm) lid (for more information see (Nunes et al., 2019) (Nunes et al., 2018)), where they were left for two hours before the experiment for acclimatation. The arena was housed in a wood box, to prevent termites from any uncontrolled stimulus, and connected by a hose to an injection chamber from where air was gently pumped into by an air pump (Fig. 2.1).

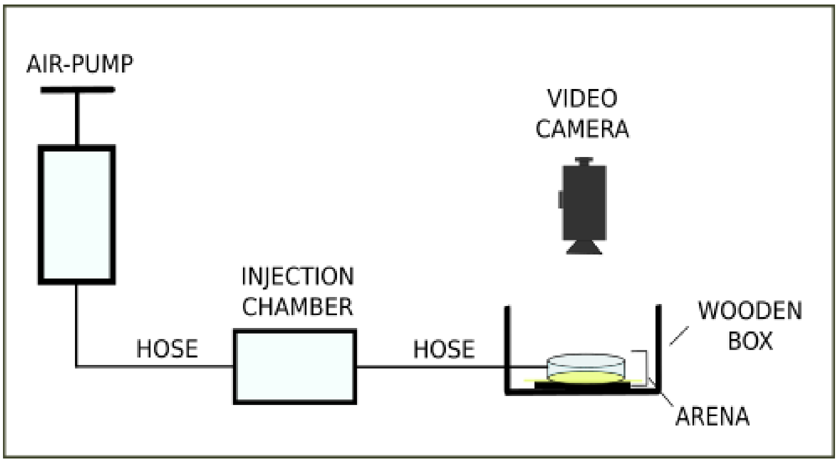

Figure 2.1: Representation of the experimental setup (Nunes et al., 2019)

The air in the injection chamber was in contact with the stimulus (Tab. 2.1) to be presented to the termites. For assays, we used air as a physical stimulus, air + paper for physicochemical stimulus and hexane and soldiers head extract (HSE) as chemical stimuli.

After the air got in contact with the stimulus in the injection chamber it was gently pumped into the experimental arena (for more information see (Nunes et al., 2019)). For the air stimulus, the injection chamber was empty, whereas for the other stimulus (air + paper, hexane and HSE) there was a clean filter paper (air + paper) or a filter paper with chemical stimulus (hexane or HSE).

Each group of termites was submitted to one of the four types of alarm and all colonies were submitted to all stimuli.

Type	Stimulus
Physical	Air
Physicochemical	Air+paper
Chemical	Hexane
Chemical	Soldier's Head Extract (HSE)

Table 2.1: list of stimulus termites were submitted to

Stimuli air and air + paper were considered as low alarm stimuli, since they are not potentially lethal for the termites. Head soldier's extract and hexane represented the most alarmer of the stimuli tested here because i) chemical stimuli were carried to termites through the air pumped passing by the filter paper, so in this case, there was an accumulative effect of the previous alarm stimuli. ii) head soldier's extract is the natural channel of termites to communicate alarm (Prestwich, 1979), then an intensive response was expected. iii) hexane is potentially lethal for insects (Ferreira-Caliman et al., 2014), by which a more defensive response was also expected in this case. We varied the dose (volume) of chemical stimuli offered for termites to had different levels of the most alarmer stimuli.

Termite responses were video-recorded by a SONY HDR-CX405 TM digital camera for 420 seconds (Fig. 2.2) and the stimulus was injected approximately at 120 s, so it would be possible to observe the termites before and after the insertion of the stimulus. There was a total of fifty eight videos.

2.4 Behavioural analysis

The videos were analysed in Boris software (Friard and Gamba, 2016). A printscreen of the video to be analysed was taken in time 0 s and, at this point, termites were marked with colored paint in *Microsoft Paint* (1985) software. With this procedure we ensured that each individual was observed just once. To avoid different interpretations of the

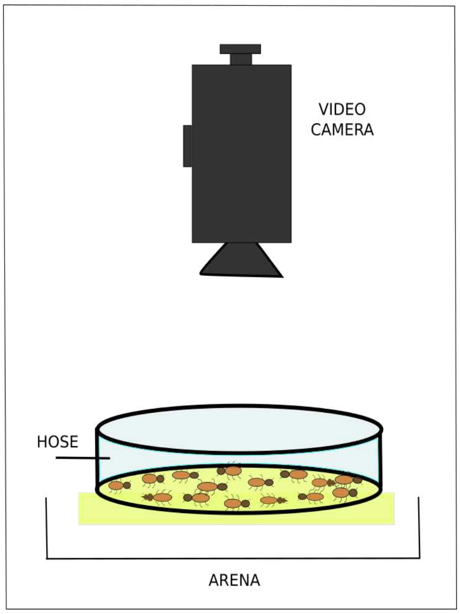

Figure 2.2: Representation of the recording of termites

distinct behaviours, the videos were analysed by only one observer.

We analysed the videos for 240 s, totalling 7200 video frames observed per assay. In total, 108.000 video frames have been analysed.

From the information gathered with Boris software we constructed an ethogram. For that, each termite received an identity, so it was possible to identify which termite was performing certain behaviour. Behaviours were defined (see Tab. 2.2) based in previous works (Hugo et al., 2020).

For the analysis, situations before and after stimulus insertion were considered.

Individuals who died during acclimatation period, that is, before the injection of the stimulus, were excluded of the analysis.

2.5 Shannon index calculation

Shannon's index is accessed measuring the diversity of the parts that compound the system, from which is possible to infer the amount of information circling (Mikhailovsky and Levich, 2015). The greater the disorder, the larger the shannon's index and the larger the diversity of system's information (Mikhailovsky and Levich, 2015).

Here, we access the disorder of the system measuring the Shannon's diversity index

Behaviour	Definition
Antennation plate	To antennae the plate (lateral or above)
Antennation termite	To antennae another termite
Attack	To attack another termite (aggressive behaviour)
Bypass	To pass by another termite and continue walking/running in the same direction
Dead	To be dead
Eat	To eat the filter paper
Explore walk	To walk around the arena
Explore run	To run around the arena
Grooming mate	To get or to give groom to a nestmate
Grooming plate	To groom the plate
Grooming self	To groom itself
Obs perdida	When is impossible to see the termite or what it is doing
Poop	To defecate
Reverse	To pass by another termite and change the direction of the walk/run
Rest	To rest, be without movement for a while
Tropholaxy buccal	To exchange substances via oral
Tropholaxy proctodeal	To exchange substances via anus
Vibration	To vibrate the body (jerk or drum)

Table 2.2: List of the behaviours observed in Boris software

of the number of different behaviours performedby individuals before and after the injection of the stimuli. For the interpretation, we followed the same logic, the larger the Shannon's index, the greater diversity of behaviours and the greater the disorder of the system.

It was obtained by the summation of the product of relative frequency (p_i) of each behaviour and its natural logarithm (ln).

$H' = \sum_{i=1}^{S} p_i ln p_i$

Where H' is the Shannon's index; S is the number of distinct behaviours ; and p_i is the frequency the number of thimes of a given behaviour was recorded, relative to the total number of observations.

2.6 Statistical analysis

Data was analysed using the Shannon's index as response variable and the variables: type of stimulus, point of stimulus insertion (two levels: before and after) and nest (block variable) as explanatory variables. Nests were analysed as a block variable to make sure that possible Shannon's index variations would be related to stimulus properties rather than for nests characteristics. Models were performed using a GLM under Gaussian error distribution. We performed the analysis following the sequence:

First, we tested if the Shannon's index of behaviours exhibited before the insertion of the stimulus was modified after insertion. This was performed by each stimulus separately.

For the second step, we tested if Shannon's index of behaviours differed according to the type of stimulus. Here only the data after the injection of the stimulus were considered.

Finally, we tested if Shannon's index varied as a function of the volume of chemical stimuli offered to the termites. For this analysis we used the data from hexane and HSE. Only the data after the injection were considered.

Model selection was performed using the Akaike's information criterion (AICc). Only models with substancial empirical support were considered ($\Delta(AIC) \leq 2.0$).

A contrast analysis was made to verify if stimuli types differed from each other regarding their effect on Shannon's Index.

Chapter 3

Results

3.1 Signals/cues trigger disorder

We observed an increase of Shannon's index after the insertion of all stimuli. Which would denote an increase in the diversity of behaviours. That is to say, the level of behavioural "disorder" increased after termites were exposed to any type of stimulus (Tab. 3.1, Fig. 3.1).

Id	(Intercept)	Nest	Point of Insertion	df	logLik	AICc	Δ	weight
4	1.18	+	+	17	-532.0	1098.4	0.0	1.00
3	1.17		+	3	-582.3	1170.7	72.3	0.00

Table 3.1: Models with substantial empirical evidence ($\Delta \leq 2.\ 0$) predicting the effect of the insertion of the alarm stimulus on the shannon's index. Column headings: id = model identification, intercept = model intercept, nest = id of the nest from which individuals were extracted, point of insertion= point at which the stimulus was injected (before and after). df = degree of freedmon, AICc = second-order Akaike information criterion, Δ = AICc difference between the model in concern and the best model, weight = Akaike weight.

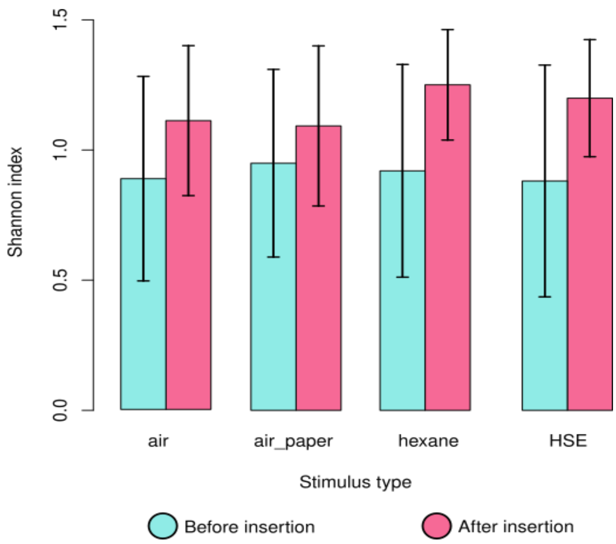

Figure 3.1: Bar-plot of Shannon's index regarding to the point of the insertion of stimulus. Shannon's index increased after the insertion of the stimulus regarding the condition before. The same pattern was found for all the type of stimuli. In blue shannon's index before the stimulus. In pink, after it's insertion.

3.2 Biochemical signals trigger more disorder than physical signals

The disorder caused by the stimuli was more noticeable for stimuli of chemical or biochemical nature (hexane and HSE) than for stimuli of physical and physicochemical types(Tab. 3.2 Fig. 3.2). Biochemical alarms Shannon's index (and hence disorder) triggered by chemical and biochemical stimuli was higher than this index for termites exposed to physical and physicochemical stimuli. Futhermore, stimuli air and air + paper did not differ statistically, while stimuli hexane and soldier head extract (HSE) did differ between each other (Tab. 3.3. So, statistically there were three groups of stimuli (air/air + paper, hexane and extract) (Fig. 3.2). In addition, in all analysis, nest was a significant variable.

Id	(Intercept)	Nest	Stimulus	df	logLik	AICc	Δ	weight
4	1.18	+	+	19	-15.6	70.2	0.0	1.00
3	1.11		+	5	-53.7	117.4	47.3	0.00

Table 3.2: Models with substantial empirical evidence (Δ ≤2. 0) predicting the effect of the insertion of the alarm stimulus on the shannon's index. Column headings: id = model identification, intercept = model intercept, nest = id of the nest from which individuals were extracted, stimulus = type of stimulus injected (before and after). df = degree of freedom, AICc = second-order Akaike information criterion, Δ = AICc difference between the model in concern and the best model, weight = Akaike weight.

Id	df	AICc
all stimuli separareted	19	69.22520
air+air+paper	18	67.49029
air+air+paper+HSE	17	88.64595
HSE+hexane	17	70.40049

Table 3.3: Models with substantial empirical evidence (smallest value of AICc) predicting the best grouping model. Column headings: Id = model identification, df = degree of freedom, AICc = AICc difference between the model in concern and the best model.

3.3 Within a given stimulus, higher doses trigger order

Shannon's index decreased with the increments in the volume of the chemical and biochemical stimuli denoting a move towards order. However, we found that the stimulus hexane triggered a higher alarm response than the HSE stimulus. In other words, the diversity of behaviours of the group exposed to HSE was smaller than the group exposed to hexane (Tab. 3.4, Fig. 3.3).

Id	(Intercept)	Dose	Stimulus	df	logLik	AICc	Δ	weight
4	1.28	-0.001	+	4	48.3	-88.5	0.0	0.71
3	1.25		+	3	45.9	-85.7	2.8	0.18

Table 3.4: Models with substantial empirical evidence (Δ ≤2. 0) predicting the effect of the dose of the chemical stimuli on the shannon's index. Column headings: id = model identification, intercept = model intercept, dose = the volume (μL) of chemical stimulus injected , stimulus = type of chemical stimulus (hexane and HSE) injected. df = degree of freedmon, AICc = second-order Akaike information criterion, Δ = AICc difference between the model in concern and the best model, weight = Akaike weight.

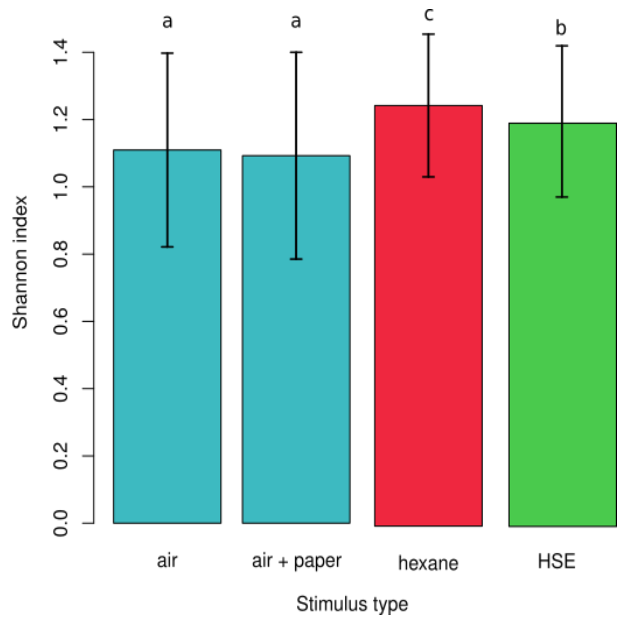

Figure 3.2: Bar-plot of Shannon's index regarding the distinct types of stimuli. Stimuli air and air + paper in blue were not statistically different. Stimulus hexane, in red, and HSE, in green, were different among them and also resgarded to the stimuli air and air + paper.

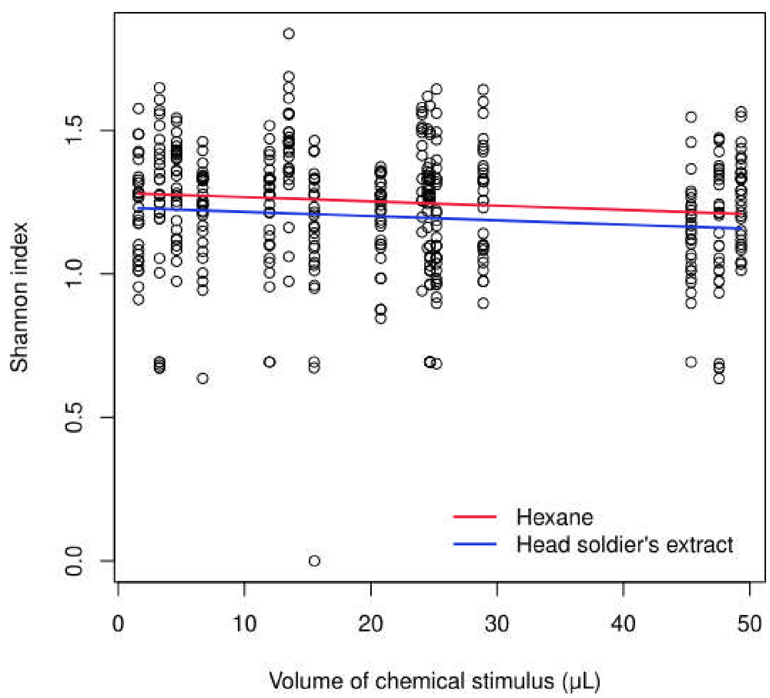

Figure 3.3: Shannon's index as a function of the volume (μL) of chemical stimulus. The circles represent each one of the individuals analysed. The red line represents shannon's index varying according the hexane doses whereas the blue line represents shannon's index varying in relation to soldier's head extract doses.

Chapter 4

Discussion

It is frequently expected that individuals increase their behaviour's diversity when under an alarm situation, generating system's disorder. Accordingly, the diversity of behaviours here observed, increased after the insertion of the stimuli, regardless its identity: physical, chemical, or biological origin (Fig. 3.1). However, this increment in behaviour's diversity was more expressive for chemical stimuli than for physical and physochemical types (Fig. 3.2).

Assuming that purely physical alarm signals (air) would convey milder messages than alarm signals of clear biological origin (HSE), one could argue that this result supports our hypothesis that increments in alarm intensity can switch order and disorder in the behavioural repertories of animal groups.

What seems to be happening is that mild alarm stimulated a few individuals only, leading to increments in the beavioural repertory of the group, because new behaviours arise as previous ones persist. Mild stimulation of the colony under alarm has been already observed in *Macrotermes subhyalinus*, whose individuals tended to keep their regular activities under threat (Kettler and Leuthold, 1995).

On its turn, stronger alarms would have stimulated the majority of the group members, aligning their behaviour hence limiting behavioural repertories and behavioural diversity. This seems to be in line with the results found by Reinhard and Clément (2002) studying Reticulitermes termites: under alarm, these termites presented a more variable range of behavious than when in a regular scenario.

The variation in behavioural diversity may be strongly related to the proportion of individuals recruited due to the intensity of alarm signal. The reason for that relies on the dependency of alarm responses on alarm intensity (Rosengaus et al., 1999). That is, the more intense is the alarm signal, the more individuals are recruited (Cristaldo et al., 2015).

Therefore, it is expected that in higher alarm intensities individuals exhibit a higher alarm response, which could lead to disorder. That is the possible reason why hexane promotes an increase in behaviours diversity. Pure hexane is potentially lethal for insects (Ferreira-Caliman et al., 2014), therefore it triggered a magnified alarm response leading individuals to a lower level of order.

However, the stronger the signal, the quicker the message travels across a group, causing individuals to quickly align their behaviour (DeSouza et al., 2001) (Lemonik Arthur, 2013). This would promote a decrease in behaviour's diversity and an increase of order. That is probably the reason why increments in the volume of the chemical stimuli (hexane and HSE) triggered a decrease in the Shannon's index (Tab. 3.4 Fig. 3.3). Thus, individuals performed a lower diversity of behaviours in higher doses of chemical stimuli, leading to a less disordered scenario.

Futhermore, this copying pattern may have adaptive value because it can lead to herd effect (Kelley et al., 1965) (Dias et al., 2012), which may promote an organised escaping behaviour (Wang et al., 2016). Under high alarm stimuli, however, herd effect can cause more demages than goods (Altshuler et al., 2005), since it may lead to path blockings. High alarm stimuli triggers an increase in individual's speed (personal observation and (Cristaldo et al., 2015)) as well as an increase in the number of individuals recruited to the alarm area. That may promote loss of order hence high velocity in crowds may lead to uncoordinated responses (Helbing et al., 2002).

Since information measured by Shannon's index refers to the degree of uncertainty in a system, higher index value do not mean either information gain or higher transissibility. Transmission of information is enhanced when there is a balance between signal's diversity and redundancy (McCowan et al., 1999). Therefore, it is more plausible that information reaches the receiver under HSE alarm stimulus than under hexane stimulus. This also could explain why order at individual scale was higher under HSE than under hexane stimulus.

Additionally, we found that the origin of collected termites (nest) had an effect on the pattern observed. However, individuals from all the nests increased the Shannon's index after the insertion of the stimuli, variation were related to the magnitude of the response of each nests. Then, individuals of some nests showed a significant increase in their response while others had a moderate response.

Chapter 5

Conclusion

Our results suggest that, alarm intensity modulates order at individual scale in social groups. That is probably due to recruitment rate. Under low alarm, at individual scale, some disorder is observed, since we have individuals performing regular activities plus only a few individuals being recruited and therefore a few individuals responding to the new stimulus. When there is an increase in alarm intensity, an increase in disorder is observed, since we have more individuals being recruited, hence more individuals responding to the alarm. However, if the stimulus is too strong, more and more individuals are recruited and most individuals are stimulated by the same stimulus, so an orderly response is observed.

Our work brings a new view in social animals studies. It focus on how social animals behave in an individual scale under different alarm situations. It elucidates that the way an individual behaves under alarm influences the other individuals at the social group, which may promote changes in group order. It remais to be tested whether even more intense alarms, such as risk of predation, would interfere in cohesion, thus returning individuals to their idiosyncratic behaviour patterns.

Bibliography

Altshuler, E., Ramos, O., Núñez, Y., Fernández, J., Batista-Leyva, A., and Noda, C. (2005). Symmetry breaking in escaping ants. *The American Naturalist*, 166(6):643–649.

Bliss, J. P., Gilson, R. D., and Deaton, J. E. (1995). Human probability matching behaviour in response to alarms of varying reliability. *Ergonomics*, 38(11):2300–2312.

Cristaldo, P. F., Jandák, V., Kutalová, K., Rodrigues, V. B., Brothanek, M., Jiříček, O., DeSouza, O., and Šobotník, J. (2015). The nature of alarm communication in constrictotermes cyphergaster (blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. *Biology Open*, 4(12):1649–1659.

DeSouza, O., Miramontes, O., Santos, C., and Bernardo, D. (2001). Social facilitation affecting tolerance to poisoning in termites (insecta, isoptera). *Insectes Sociaux*, 48(1):21–24.

Dias, C., Sarvi, M., and Shiwakoti, N. (2012). Intersecting and merging pedestrian crowd flows under panic conditions: insights from biological entities. In *35th Australasian Transport Research Forum (ATRF)*, pages 13–20. Perth, Western Australia Australia.

Evans, T., Inta, R., Lai, J., and Lenz, M. (2007). Foraging vibration signals attract foragers and identify food size in the drywood termite, cryptotermes secundus. *Insectes Sociaux*, 54(4):374–382.

Ferreira-Caliman, M. J., Andrade-Silva, A. C. R., Guidetti-Campos, M. C., Turatti, I. C. C., do Nascimento, F. S., and Lopes, N. P. (2014). A non-lethal spme method for insect cuticular analysis by gc-ms. *Analytical Methods*, 6(21):8823–8828.

Franks, N. R. and Richardson, T. (2006). Teaching in tandem-running ants. *Nature*, 439(7073):153–153.

Friard, O. and Gamba, M. (2016). Boris: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7(11):1325–1330.

Grüter, C. and Farina, W. M. (2009). The honeybee waggle dance: can we follow the steps? *Trends in Ecology & Evolution*, 24(5):242–247.

Helbing, D., Farkas, I. J., Molnar, P., and Vicsek, T. (2002). Simulation of pedestrian crowds in normal and evacuation situations. *Pedestrian and evacuation dynamics*, 21(2):21–58.

Hugo, H., Cristaldo, P. F., and DeSouza, O. (2020). Nonaggressive behavior: A strategy employed by an obligate nest invader to avoid conflict with its host species. *Ecology and evolution*, 10(16):8741–8754.

Jacobsen, H. and Stabell, O. (1999). Predator-induced alarm responses in the common periwinkle, littorina littorea: dependence on season, light conditions, and chemical labelling of predators. *Marine Biology*, 134(3):551–557.

Kelley, H. H., Condry Jr, J. C., Dahlke, A. E., and Hill, A. H. (1965). Collective behavior in a simulated panic situation. *Journal of Experimental Social Psychology*, 1(1):20–54.

Kettler, R. and Leuthold, R. (1995). Inter-and intraspecific alarm response in the termite macrotermes subhyalinus (rambur). *Insectes sociaux*, 42(2):145–156.

Lemonik Arthur, M. M. (2013). Emergent norm theory. *The Wiley-Blackwell Encyclopedia of Social and Political Movements*.

Maschwitz, U. (1967). Alarm substances and alarm behavior in social insects. *Vitamins & Hormones*, 24:267–290.

McCowan, B., Hanser, S. F., and Doyle, L. R. (1999). Quantitative tools for comparing animal communication systems: information theory applied to bottlenose dolphin whistle repertoires. *Animal behaviour*, 57(2):409–419.

Mikhailovsky, G. E. and Levich, A. P. (2015). Entropy, information and complexity or which aims the arrow of time? *Entropy*, 17(7):4863–4890.

Nunes, L., Fellipe Cristaldo, P., Sérgio Silva, P., Bonato Felix, L., Miranda Ribeiro, D., and DeSouza, O. (2019). Dataset on substrate-borne vibrations of constrictotermes cyphergaster (blattodea: Isoptera) termites. *Data*, 4(2):87.

Nunes, L. F., Roxinol, J. A., Cristado, P. F., Marinho, R., and DeSouza, O. (2018). The use of tympanic arena as an alternative for behavioral vibroacoustic essays in termites (blattodea: Isoptera). *Sociobiology*, 65(1):101–107.

Prestwich, G. D. (1979). Chemical defense by termite soldiers. *Journal of Chemical Ecology*, 5(3):459–480.

Reinhard, J. and Clément, J.-L. (2002). Alarm reaction of european reticulitermes termites to soldier head capsule volatiles (isoptera, rhinotermitidae). *Journal of Insect behavior*, 15(1):95–107.

Rosengaus, R., Jordan, C., Lefebvre, M., and Traniello, J. (1999). Pathogen alarm behavior in a termite: a new form of communication in social insects. *Naturwissenschaften*, 86(11):544–548.

Speedie, N. and Gerlai, R. (2008). Alarm substance induced behavioral responses in zebrafish (danio rerio). *Behavioural brain research*, 188(1):168–177.

Wang, C., Henderson, G., Gautam, B. K., Chen, J., and Bhatta, D. (2016). Panic escape polyethism in worker and soldier coptotermes formosanus (isoptera: Rhinotermitidae). *Insect science*, 23(2):305–312.

Chapter 6

Appendix

Before statistical analysis, Shannon's index was calculated with the use of *Microsoft Excel* (1987) software. It was calculated from the sum of the products of relative frequencies (p_i freqRelative) and its natural logarithm (lnp_i lnfreqrelative), as explicited in the formula below:

$H' = \sum_{i=1}^{S} p_i ln p_i$

To obtain freqRelative and lnfreqrelative, the absolute frequency (freqAbs) and its summation (freqSum) were calculated (see Tab. 6.1).

Calculus	**How it was calculated**
freqAbs	number of times a behaviour appeared per individual
freqSum	$= sum(freqAbs)$
freqRelative	$= freqAbs/freqSum$
lnfreqrelative	$= LN(freqRelative)$

Table 6.1: list of how freqAbs, freqRelative and lnfreqrelative were obtained.

Shannon's index was calculated for all individuals from all nests, submitted to all stimuli. The column readers of the spreadsheet (Fig. 6.1 for more details see 10.5281/zenodo.5120999) used are:

1. nest: the nest where the termites analysed belong to
2. stimulus: the type of stimulus (cited below) the termites of a given nest are submitted to air = air, air_paper = air plus paper, hexane = air plus paper plus a volume of hexane, termite: air plus paper plus the soldiers' head extract prepared using hexane.
3. dose: the amount of the dose in microliters of chemical stimuli (hexane and termite (extract of hexane and soldier's head gland)) in the injection chamber.
4. dosePheromone: the number of soldier´s head glands used to do the extract.
5. subject: each one of the termites submitted to the experiment
6. behaviour: number of behaviours performed by the individuals during each phase: before and after the injection of each stimulus.
7. status: the condition of the behaviour, where START = when the behaviour starts and STOP = when the behaviour stops.
8. situation: the moment where the termites are submitted to regarding the injection of the stimulus, where: before = before the injection of the stimulus, after = after the injection of the stimulus.
9. caste: the caste from where the termite belongs to, where s = soldier and w = worker.
10. freqAbs: absolut frequency.
11. freqSum: sum of the absolut frequencies ordered in before and after the insertion of the stimulus.
12. freqRelative: relative frequency.

13. lnfreqrelative: natural logarithm of the variable freqRelative.

1	nest	stimulus	dose	dosePheromone	subject	behavior	behavior_id	status	situation	caste	freqAbs	freqSum	freqRelative	lnfreqrelative
2	n01	air	0	0	soldier_01	exploreWalk	a	START	before	s	2	5	0.4	-0.916290731874155
3	n01	air	0	0	soldier_02	exploreWalk	a	START	before	s	1	3	0.333333333333333	-1.09861228866811
4	n01	air	0	0	soldier_03	exploreWalk	a	START	before	s	8	19	0.421052631578947	-0.864997437486605
5	n01	air	0	0	worker_01	exploreWalk	a	START	before	w	2	2	1	0
6	n01	air	0	0	worker_02	exploreWalk	a	START	before	w	4	8	0.5	-0.693147180559945
7	n01	air	0	0	worker_03	antennationPlate	b	START	before	w	4	11	0.363636363636364	-1.01160091167848
8	n01	air	0	0	worker_04	rest	c	START	before	w	1	6	0.166666666666667	-1.79175946922805
9	n01	air	0	0	worker_05	exploreWalk	a	START	before	w	1	4	0.25	-1.38629436111989
10	n01	air	0	0	worker_06	rest	c	START	before	w	3	14	0.214285714285714	-1.54044504094715
11	n01	air	0	0	worker_07	rest	c	START	before	w	3	5	0.6	-0.510825623765991
12	n01	air	0	0	worker_08	exploreWalk	a	START	before	w	7	14	0.5	-0.693147180559945
13	n01	air	0	0	worker_09	exploreWalk	a	START	before	w	1	2	0.5	-0.693147180559945
14	n01	air	0	0	worker_10	exploreWalk	a	START	before	w	2	4	0.5	-0.693147180559945
15	n01	air	0	0	worker_11	exploreWalk	a	START	before	w	7	12	0.583333333333333	-0.538996500732688
16	n01	air	0	0	soldier_02	rest	c	START	before	s	1	3	0.333333333333333	-1.09861228866811
17	n01	air	0	0	worker_09	rest	c	START	before	w	1	2	0.5	-0.693147180559945
18	n01	air	0	0	worker_05	rest	c	START	before	w	2	4	0.5	-0.693147180559945
19	n01	air	0	0	worker_06	exploreWalk	a	START	before	w	6	14	0.428571428571429	-0.847297860387203
20	n01	air	0	0	soldier_01	antennationPlate	b	START	before	s	1	5	0.2	-1.6094379124341
21	n01	air	0	0	soldier_02	antennationPlate	b	START	before	s	1	3	0.333333333333333	-1.09861228866811
22	n01	air	0	0	worker_03	exploreWalk	a	START	before	w	5	11	0.454545454545455	-0.788457360364269
23	n01	air	0	0	worker_04	exploreWalk	a	START	before	w	3	6	0.5	-0.693147180559945
24	n01	air	0	0	worker_04	antennationTermite	d	START	before	w	1	6	0.166666666666667	-1.79175946922805
25	n01	air	0	0	soldier_03	antennationPlate	b	START	before	s	5	19	0.263157894736842	-1.33500106673234
26	n01	air	0	0	worker_10	vibration	e	START	before	w	1	4	0.25	-1.38629436111989
27	n01	air	0	0	soldier_01	rest	c	START	before	s	2	5	0.4	-0.916290731874155
28	n01	air	0	0	worker_06	antennationPlate	b	START	before	w	2	14	0.142857142857143	-1.94591014905531
29	n01	air	0	0	worker_08	antennationPlate	b	START	before	w	7	14	0.5	-0.693147180559945
30	n01	air	0	0	worker_03	rest	c	START	before	w	2	11	0.181818181818182	-1.70474809223842

Figure 6.1: Spreadsheet used to calculate Shannon’s index.

6.1 Statistical analysis

6.1.1 Creating workspace

In the dataset bellow (fully available at: 10.5281/zenodo.5120999) the column headers are:

1. nest: the nest where the termites analysed belong to
2. stimulus: the type of stimulus (cited below) the termites of a given nest are submitted to air = air, *air_paper* = air plus paper, hexane = air plus paper plus a volume of hexane, termite: air plus paper plus the soldiers' head extract prepared using hexane.
3. dose: the amount of the dose in microliters of chemical stimuli (hexane and termite (extract of hexane and soldier's head gland)) in the injection chamber.
4. dosePheromone: the number of soldier´s head glands used to do the extract.
5. subject: each one of the termites submitted to the experiment
6. behaviour: number of behaviours performed by the individuals during each phase: before and after the injection of each stimulus.
7. status: the condition of the behaviour, where START = when the behaviour starts and STOP = when the behaviour stops.
8. situation: the moment where the termites are submitted to regarding the injection of the stimulus, where: before = before the injection of the stimulus, after = after the injection of the stimulus.
9. caste: the caste from where the termite belongs to, where s = soldier and w = worker.
10. shannon: Shannon index per individual.

```
> setwd("~/Documents/Tese/tese_pratica/table_complete/Cálculos shannon/índice de shannon por indivíduo/Cálculo da frequência d
> dados <- read.csv("table_calculus_ready03.csv", header = TRUE, sep = ",")
> head(dados, n = 5)

  nest stimulus dose dosePheromone    subject    behavior behavior_id status
1  n01      air    0             0 soldier_01 exploreWalk           a  START
2  n01      air    0             0 soldier_02 exploreWalk           a  START
3  n01      air    0             0 soldier_03 exploreWalk           a  START
4  n01      air    0             0  worker_01 exploreWalk           a  START
5  n01      air    0             0  worker_02 exploreWalk           a  START
  situation caste   shannon
1    before     s     1.054920
2    before     s     1.098612
3    before     s     1.298418
4    before     w     0.000000
5    before     w     1.213008
```

6.1.2 Subsets of data set

```
> before <- subset(dados, situation == "before")
> after <- subset(dados, situation == "after")
> air <- subset(dados, stimulus == "air")
> air_paper <- subset(dados, stimulus == "air_paper")
> hexane <- subset(dados, stimulus == "hexane")
> termite <- subset(dados, stimulus == "termite")
> hexaneA <- subset(after, stimulus == "hexane")
> termiteA <- subset(after, stimulus == "termite")
> chem <- subset(dados, stimulus == "hexane" & situation == "after"
+                | stimulus == "termite" & situation == "after")
> mech <- subset(dados, stimulus == "air" & situation == "after"
+                | stimulus == "air_paper" & situation == "after")
```

6.1.3 Does shannon's index change after injection of the stimulus?

```
> sort(tapply(dados$shannon, dados$situation, mean))
```

```
   before     after
0.9083108 1.1668169
```

AICc as cirterium for model selection

```
> library(MuMIn)
> options(na.action = "na.fail")
> mt1 <- glm(dados$shannon ~ dados$nest
+            + dados$situation,
+            family = gaussian)
> mt1B <- mt1
> r1Bin <- dredge(mt1B, rank = "AICc", extra = "adjR^2")
> r1Bin
```

```
Global model call: glm(formula = dados$shannon ~ dados$nest + dados$situation, fami
---
Model selection table
  (Int) dds$nst dds$stt  adjR^2 df   logLik   AICc  delta weight
4 1.184       +       + 0.31160 17 -532.030 1098.4   0.00      1
3 1.167               + 0.22090  3 -582.347 1170.7  72.28      0
2 1.054       +         0.09165 16 -649.227 1330.8 232.35      0
1 1.037                 0.00000  2 -693.590 1391.2 292.76      0
Models ranked by AICc(x)
```

Following the AICc criterium are 1 model equally probable (*delta* < 2):

```
> r1Bin[r1Bin$delta<=2]

Global model call: glm(formula = dados$shannon ~ dados$nest + dados$situation, fami
---
Model selection table
  (Int) dds$nst dds$stt adjR^2 df  logLik   AICc delta weight
4 1.184       +       + 0.3116 17 -532.03 1098.4     0      1
Models ranked by AICc(x)

> summary(get.models(r1Bin, 1)[[1]])

Call:
glm(formula = dados$shannon ~ dados$nest + dados$situation +
    1, family = gaussian)


Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.3054  -0.1719   0.0461   0.2103   1.9121


Coefficients:
```

```
                         Estimate Std. Error t value Pr(>|t|)
(Intercept)               1.183808   0.032498  36.427  < 2e-16 ***
dados$nestn02            -0.086199   0.044168  -1.952  0.05115 .
dados$nestn03             0.078642   0.044968   1.749  0.08050 .
dados$nestn04            -0.124727   0.043983  -2.836  0.00463 **
dados$nestn05            -0.007330   0.047819  -0.153  0.87818
dados$nestn06            -0.085432   0.043893  -1.946  0.05178 .
dados$nestn07            -0.003933   0.047211  -0.083  0.93362
dados$nestn08             0.007722   0.043983   0.176  0.86066
dados$nestn09            -0.009873   0.043629  -0.226  0.82099
dados$nestn10            -0.188305   0.047211  -3.989 6.94e-05 ***
dados$nestn11             0.090424   0.043803   2.064  0.03914 *
dados$nestn12             0.063904   0.043983   1.453  0.14644
dados$nestn13             0.121577   0.043803   2.776  0.00557 **
dados$nestn14            -0.065365   0.043983  -1.486  0.13744
dados$nestn15            -0.081619   0.044075  -1.852  0.06423 .
dados$situationbefore -0.257947   0.016340 -15.786  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 0.1116654)

    Null deviance: 224.45  on 1672  degrees of freedom
Residual deviance: 185.03  on 1657  degrees of freedom
AIC: 1098.1

Number of Fisher Scoring iterations: 2
```

O índice de shannon aumenta após a inserção do estímulo

6.2 The shannon index varies between treatments after the injection of the stimulus?

AICc criterium

```
> options(na.action = "na.fail")
> m2TSA <- glm(after$shannon ~ after$nest
+              + after$stimulus, family = gaussian)
> m2TSAS <- m2TSA
> r1BinSA <- dredge(m2TSAS,
+                   rank = "AICc", extra = "adjR^2")
> r1BinSA

Global model call: glm(formula = after$shannon ~ after$nest + after$stimulus, famil
---
Model selection table
  (Int) aft$nst aft$stm adjR^2 df  logLik  AICc delta weight
4 1.178       +       + 0.8173 19 -15.613  70.2  0.00      1
3 1.109               + 0.3420  5 -53.678 117.4 47.27      0
2 1.235       +         0.4642 16 -44.217 121.1 50.94      0
1 1.167                 0.0000  2 -79.062 162.1 91.98      0
Models ranked by AICc(x)

> r1BinSA[r1BinSA$delta<=2] #O unico modelo provável é o completo.

Global model call: glm(formula = after$shannon ~ after$nest + after$stimulus, famil
---
Model selection table
  (Int) aft$nst aft$stm adjR^2 df  logLik AICc delta weight
4 1.178       +       + 0.8173 19 -15.613 70.2     0      1
Models ranked by AICc(x)
```

Contrast analysis

```
> sort(tapply(predict(m2TSA),after$stimulus,mean))

air_paper       air   termite    hexane

 1.092581  1.109451  1.199270  1.250593

> #air_paper + air

>

> after$stimulusA <- recode(after$stimulus, "c('air_paper','air')='apa'")

> nairPaper <- glm(after$shannon ~ after$nest

+             + after$stimulusA,

+             family = gaussian)

> AIC(m2TSA, nairPaper) #Posso juntar ar e ar mais papel

          df      AIC

m2TSA     19 69.22520

nairPaper 18 67.49029

> ############################

> #air_paper + air + termite

>

> after$stimulusB <- recode(after$stimulus, "c('air_paper','air', 'termite')='apat

> nairPaperT <- glm(after$shannon ~ after$nest

+             + after$stimulusB,

+             family = gaussian)

> AIC(nairPaper, nairPaperT) #NÃO posso juntar ar, ar mais papel e cupim

           df      AIC

nairPaper  18 67.49029

nairPaperT 17 88.64595

> ############################

> # termite + hexane
```

```
>
> after$stimulusC <- recode(after$stimulusA, "c('termite','hexane')='th'")
> nTH <- glm(after$shannon ~ after$nest
+            + after$stimulusC,
+            family = gaussian)
> AIC(nairPaper, nTH) # três grupos: air + air papel, hexane, termite
```

```
          df      AIC
nairPaper 18 67.49029
nTH       17 70.40049
```

There are three groups: air + airPaper, hexane, termite.

6.3 Shannon's index changed as a function of the volume of chemical stimulus?

AICc criterium

```
> options(na.action = "na.fail")
> m2HS <- glm(chem\$shannon ~
+                     chem\$dose + chem\$stimulus, family = gaussian)
> m2HS1 <- m2HS
> r1BinHS <- dredge(m2HS1, rank = "AICc", extra = "adjR^2")
> r1BinHS
```

```
Global model call: glm(formula = chem\$shannon ~ chem\$dose + chem\$stimulus, famil
---
Model selection table
  (Int)  chm\$dos chm\$stm   adjR^2 df logLik  AICc delta weight
4 1.283 -0.00148       + -0.11370  4 48.296 -88.5  0.00  0.709
3 1.251                + -0.06381  3 45.889 -85.7  2.78  0.177
```

```
2 1.257 -0.00148         -0.04992  3 45.224 -84.4  4.11  0.091
1 1.225                   0.00000  2 42.850 -81.7  6.83  0.023
Models ranked by AICc(x)
```

Following the AICc criterium only one model is probable (*delta* < 2).

```
> r1BinHS[r1BinHS\$delta<=2]

Global model call: glm(formula = chem\$shannon ~ chem\$dose + chem\$stimulus, famil
---
Model selection table
  (Int)  chm\$dos chm\$stm  adjR^2 df logLik  AICc delta weight
4 1.283 -0.00148       + -0.1137  4 48.296 -88.5     0      1
Models ranked by AICc(x)
```

Summary of the model 1:

```
> summary(get.models(r1BinHS, 1)[[1]])

Call:
glm(formula = chem\$shannon ~ chem\$dose + chem\$stimulus + 1, family = gaussian)


Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.2596  -0.1386   0.0288   0.1432   0.5743


Coefficients:
                       Estimate Std. Error t value Pr(>|t|)
(Intercept)           1.2825612  0.0206860  62.001   <2e-16 ***
chem\$dose            -0.0014802  0.0006751  -2.193   0.0289 *
chem\$stimulustermite -0.0513297  0.0207067  -2.479   0.0136 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
(Dispersion parameter for gaussian family taken to be 0.0473779)

    Null deviance: 21.318  on 441  degrees of freedom
Residual deviance: 20.799  on 439  degrees of freedom
AIC: -88.592

Number of Fisher Scoring iterations: 2
```

6.4 Complementar material

Below there is a list of all behaviours observed in our experiments. They are divided by before and after the insertion of stimulus (Tab. 6.2). Differences in low and high alarm stimuli were not considered.

Before stimulus	**After stimulus**
Antennation plate	Antennation plate
Antennation termite	Antennation termite
Bypass	Bypass
Eat	Eat
Explore walk	Explore walk
Explore run	Explore run
Grooming Mate	Grooming Mate
Grooming plate	Grooming plate
Grooming self	Grooming self
Poop	-
Reverse	Reverse
Rest	Rest
Tropholaxy oral	Tropholaxy oral
Vibration	Vibration

Table 6.2: List of behaviours observed before and after the insertion of stimulus.