

UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CENTRO DE AQUICULTURA DA UNESP

**Análise Bayesiana em estudos de comportamento agressivo de
peixes**

Eurico Mesquita Noleto Filho

Jaboticabal, SP
2018

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Orientador: Dra. Eliane Gonçalves de Freitas

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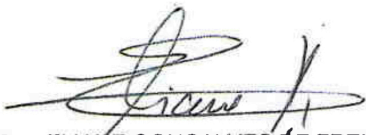
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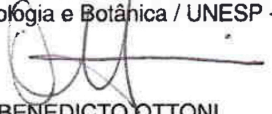
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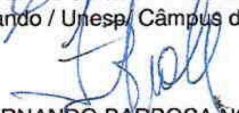
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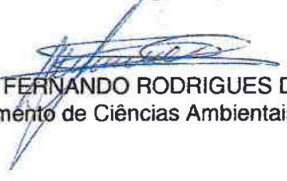
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“A menos que modifiquemos a nossa maneira de pensar, não seremos capazes de resolver os problemas causados pela forma como nos acostumamos a ver o mundo”.

Albert Einstein

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RESUMO

Este trabalho tem como objetivo testar se uma abordagem Bayesiana aprimora a análise de modelos longitudinais em estudo de comportamento agressivo em peixes. Discutimos as vantagens da análise Bayesiana ao lidar com variáveis agrupadas, resultados não estatisticamente significativos e número de réplicas utilizando o Acará Bandeira (*Pterophyllum scalare*), como estudo de caso. Além disso, em um segundo estudo com dados de quatro espécies de ciclídeo (*Pterophyllum scalare*, *Oreochromis niloticus*, *Astronotus ocellatus* e *Cichlasoma*), verificamos os diferentes tipos de viés que combinar variáveis baseando-se em critérios energéticos, pode causar na análise de dados. Por fim, em um terceiro estudo, nós construímos uma ferramenta estatística usando o pacote *Shiny* para permitir que modelos Bayesianos se tornem mais acessíveis para estudos longitudinais com agressividade. O delineamento dos dois primeiros estudos foi semelhante. Para cada espécie, 15 grupos de 3 indivíduos foram submetidos a observações diárias durante 10 minutos em 5 dias. Para o primeiro estudo, as variáveis comportamentais foram analisadas parcialmente combinadas de acordo com a intensidade do comportamento (Ataques e Displays), e completamente combinadas (soma de todas variáveis). Para o segundo, as variáveis comportamentais foram analisadas individualmente e parcialmente combinadas de acordo com a intensidade do comportamento (Ataques e Displays). As frequências de cada registro foram modeladas usando cadeias de Monte Carlo Markov. Os resultados mostram que combinar variáveis, independente do critério, pode levar a conclusões tendenciosas, pois as variáveis que estão sendo combinadas podem apresentar diferentes frequências ou padrões temporais. Além disso, essa diferença de padrão pode acontecer de forma mais clara ou mais sutil. Alterações sutis não podem ser detectadas utilizando os métodos clássicos. Os métodos Bayesianos permitem verificar a viabilidade de combinar variáveis, pois mostram de maneira exata as mudanças na probabilidade de diferença. Outrossim, fornecem uma descrição clara das mudanças mesmo quando os padrões são sutis. Os resultados também mostraram que 12 repetições, para todas as espécies, não altera as conclusões do estudo, bem como que o uso de um pequeno tamanho de amostra poderia ser mais evidente dentro dos dias sobrepostos, que inclui a estabilidade do ranking social. Por fim, a ferramenta BayesBehav é apresentada para facilitar o uso da abordagem Bayesiana em pesquisas com comportamento agressivo de peixes.

Palavras-chave: Análise Bayesiana, Comportamento Agressivo, Delineamento Longitudinal

ABSTRACT

This work aims to test if a Bayesian approach improves the analysis of longitudinal models in the study of aggressive behavior in fish. We discuss the advantages of Bayesian analysis when dealing with combined variables, non-statistically significant results and the number of replicates using the Acará Bandeira (*Pterophyllum scalare*), as a case study. In addition, in a second study with data of four species of cichlid (*Pterophyllum scalare*, *Oreochromis niloticus*, *Astronotus ocellatus* and *Cichlasoma*), we have checked in more detail the possible bias types that pooling variables based on energetic criterion, can cause in the data analysis. Finally, we have developed a statistical tool using the *Shiny package* to allow Bayesian models to become more accessible for aggressive longitudinal studies. We have adopted a similar experimental design for the first two studies. For each species, 15 groups of 3 subjects were submitted to daily observations for 10 minutes, in 5 days. For the first study, the behavioral variables were analyzed partially combined according to the intensity of the behavior (Attacks and Displays) and completely combined (sum of all variables). For the second, the behavioral variables were analyzed individually and partially combined according to the intensity of the behavior (Attacks and Displays). The frequencies of each record were modeled using Monte Carlo Markov chains. The results show that combining variables, regardless of the criterion, can lead to biased conclusions since the variables that are being combined have different frequencies or temporal patterns. In addition, this difference in pattern can happen more clearly or more subtly. Subtle changes cannot be detected using classical methods. Bayesian methods allow verifying the feasibility of combining variables, as they accurately show changes in the probability of difference. Also, they provide a clear description of the changes even when the temporal patterns are subtle. In addition, the results showed that twelve replicates, for all species, does not change the study's conclusions, as well as that the use of a small sample size could be more evident within the overlapping days, which includes the social rank stability. Finally, the BayesBehav tool is presented as a suitable tool to facilitate the use of the Bayesian approach in aggressive fish behavior research.

Keywords: Aggressive behavior, Bayesian analysis, Longitudinal design.

CAPÍTULO I

INTRODUÇÃO GERAL

O crescimento contínuo de indústrias, como à aquicultura e pesca, torna vital pesquisas científicas sobre o bem-estar de peixes cultivados e selvagens. Em particular, várias espécies de peixes interagem de forma agressiva para criar uma hierarquia de dominância e defender o território. Compreender os comportamentos agressivos pode beneficiar a gestão de peixes, mas ainda é necessário que seja superada a lacuna entre modelos matemáticos e dados experimentais. De fato, diferentes problemas surgem quando dados de comportamento são analisados devido à sua alta variabilidade e baixa frequência dos comportamentos individuais. Neste sentido, discutimos e propomos novas abordagens e ferramentas com o intuito de aprimorar a análise de dados de comportamentos agressivos. Estes são contextualizados nos tópicos a seguir.

Limitações na análise de dados

Em estudos com comportamento agonístico de peixes, a variável resposta é usualmente analisada de maneira quantitativa através da frequência de unidades comportamentais que caracterizam a agressividade (e.g. Todd et al. 2008, Kania 2012, Svensson et al. 2012). No entanto, a definição dessas unidades é feita de maneira qualitativa nos etogramas. Estes são inventários dos comportamentos usualmente presentes em uma espécie (Sakamoto et al. 2009). Geralmente esses comportamentos são utilizados como variáveis nos estudos comportamentais (e.g. Haller & Wittenberger 1987, Gomez-Laplaza & Morgan 1993, Werneyer et al. 2002). Alguns exemplos típicos dessas variáveis são ataques frontais, ameaças laterais e ondulações. Estas podem ser usadas de três maneiras: (i) Individualmente, quando nenhum tipo de combinação é feita nas variáveis (e.g. Oliveira et al. 2011); (ii) Parcialmente combinadas, quando agregadas baseando-se na intensidade do comportamento agressivo (e.g. Haller & Wittenberger 1987, Gomez-Laplaza & Morgan 1993, Werneyer et al. 2002); (iii) Totalmente combinadas, quando todos os eventos agonísticos são agregados em uma única variável (e.g. Gonçalves-de-Freitas et al. 2008, Maan et al. 2001, Ros et al. 2006). No entanto, entre essas classificações, a parcialmente combinada é a mais ambígua na literatura, devido à inconsistência em sua denominação. Alguns exemplos são a agressão explícita e contida (do inglês, *overt and restrained aggression*) (e.g. Desjardins et al. 2012,

Balzarini et al. 2014), os ataques de alta e baixa intensidade (do inglês, *high and low intensity attacks*) (e.g. Gomez-Laplaza & Morgan, 1993, Werneyer et al., 2002, Gonçalves-de-Freitas & Mariguela 2006), os ataques agonísticos assimétricos de alta intensidade (do inglês, *Asymmetric high-intensity agonistic attacks*), entre outros (e.g. Galhardo et al., 2008). Apesar da denominação, essas classificações têm algo em comum, todas elas são baseadas nos custos energéticos dos comportamentos agonísticos (e.g. Gomez-Laplaza & Morgan 1993, Ros et al., 2006, Arnold & Taborsky 2010). Embora a classificação das variáveis individuais de acordo com seus custos energéticos seja plausível, nenhum critério matemático foi adotado para combinar essas variáveis. Na verdade, combinar variáveis pode gerar um viés na análise de dados, especialmente quando as variáveis que foram combinadas apresentam padrões temporais distintos (Dearing et al. 2001). Portanto, é possível que exista um problema qualitativo na classificação dessas variáveis.

Além disso, algumas limitações dos dados de comportamentos agressivos podem trazer problemas quantitativos para as análises de dados. Dentre elas, destacamos: (i) as repetições são difíceis de produzir; (ii) a forma como estes comportamentos ocorrem é bastante variável dentro dos indivíduos e; (iii) o desenho experimental é longitudinal (isto é, diz respeito ao mesmo indivíduo ao longo do tempo) (Dow 1976). O uso da abordagem clássica para lidar com a essas limitações pode se tornar problemático pois impossibilita atender suposições paramétricas clássicas como normalidade, homocedasticidade e esfericidade. Além disso, essa abordagem utiliza como medida de probabilidade o “valor-p” (do inglês, *p-value*). Estes medem a probabilidade de um resultado extremo quando a mesma experiência é repetida várias vezes. A utilização do valor-p é criticada pois essa abordagem ignora resultados não significativos importantes na conclusão de estudos (Gelman 2013, Ludwig 2005, Pitak-Arnnop 2010). Por fim, temos viés das comparações múltiplas, usualmente utilizadas como testes de pós-hoc nas abordagens clássicas (e.g. Terleph 2004, Carvalho et al. 2012). Quando estas são implementadas, a probabilidade de um pesquisador concluir erroneamente que há pelo menos um efeito estatisticamente significativo em um conjunto de testes aumenta de forma aditiva a cada teste (Gelman et al. 2012).

Nesse sentido, métodos Bayesianos serão testados como uma alternativa para aprimorar a análise de dados em estudos com interações agonísticas de peixes. Esse método vem crescendo em popularidade em áreas como ciências médicas, psicologia, ecologia e pesca - e.g. Alterovitz et al. 2007, Lee & Wagenmakers 2003, McCarthy 2007, Kinas & Andrade 2007. Algumas pesquisas já demonstraram que esses métodos são importantes para estudos com comportamento animal (McNamara & Houston 1980, McNamara & Olsson 2006). Contudo, ainda é pouco usado em estudos experimentais com comportamento agressivo de peixes.

O que é a análise Bayesiana?

É uma análise fundada pelo Reverendo Thomas Bayes (que originou o nome da análise), a qual possibilita descrever a incerteza de parâmetros desconhecidos de forma probabilística. A análise trata todos os parâmetros como quantidades aleatórias tornando possível este tipo de mensuração. Um atributo particular da análise Bayesiana é a capacidade de combinar o conhecimento prévio (Priori) a novos dados por meio de um modelo que produz um resultado posterior (McCarthy 2007). O conhecimento prévio pode ser algo não informativo, que não afeta os resultados (Jeffreys 1961), ou dados que ajudam na obtenção de uma melhor estimativa.

$$Priori + dados \xrightarrow{\text{modelo}} Posterior \text{ (adaptado de } \underline{\text{McCarthy et al. 2007}})$$

Os modelos Bayesianos permitem criar uma cadeia de dependências entre os parâmetros para modelos longitudinais (modelos hierárquicos) (Kruschke & Vanpaemel 2015). Estes possibilitam estimar separadamente a variância em observações de um mesmo indivíduo ao longo do tempo e entre indivíduos diferentes. Desse modo, a distribuição posterior conjunta para esse modelo pode ser descrita por:

$$p(\theta_z, \dots, \theta_{z-1}, \sigma^2[e] | \mu, \sigma^2[z] | x) \propto p(\theta_z | \mu, \sigma^2[z]) p(\sigma^2[e]) p(\mu) p(\sigma^2[z])$$

Onde:

θ_z é média da variável mensurada em cada intervalo de tempo do período do estudo. μ é a média global de todos intervalos de tempo do período de estudo. $\sigma^2_{[z]}$ é a variância entre todos intervalos de tempo do período de estudo. $\sigma^2_{[e]}$ é a variância residual da variável medida.

Por que usar a análise Bayesiana?

Como supracitado, a análise Bayesiana têm suas vantagens evidenciadas em diversas áreas científicas. Uma dessas vantagens é possuir um paradigma estatístico diferente que possibilita medir a incerteza de parâmetros desconhecidos. Esse paradigma difere do clássico, no qual a probabilidade é definida como o limite da frequência relativa de dados observados em uma experiência se o mesmo procedimento de coleta e análise de dados fosse implementado várias vezes (McCarthy 2007). Por esta razão, em uma abordagem clássica, não podemos fornecer a probabilidade de diferença exata entre dois ou mais grupos de dados, mas a probabilidade de se obter um valor extremo ao repetir um experimento (Figura 1A). Para o método Bayesiano medir a incerteza de parâmetros desconhecidos permite calcular e inferir probabilidades exatas entre grupos (Figura 1B). Dessa forma, até os dados não significativos podem ser levados em consideração nas análises. Além disso, esse tipo de inferência pode ajudar a medir o impacto que agregar variáveis pode ter na análise de dados, pois possibilita mensurar de maneira exata o quanto a probabilidade de diferença entre dois grupos combinando ou não variáveis, muda.

Outra vantagem da análise Bayesiana é a forma multi-nível em que as comparações entre grupos são feitas, o que possibilita examinar as distribuições posteriores sob diferentes perspectivas ou margens (Kruschke, 2015). Portanto, este método é mais adequado para lidar com problemas de comparações múltiplas, pois ele altera as estimativas pontuais e seus intervalos, tornando-os próximos em vez de aumentar os intervalos de confiança como nas correções de estimativas clássicas (Gelman et al. 2012).

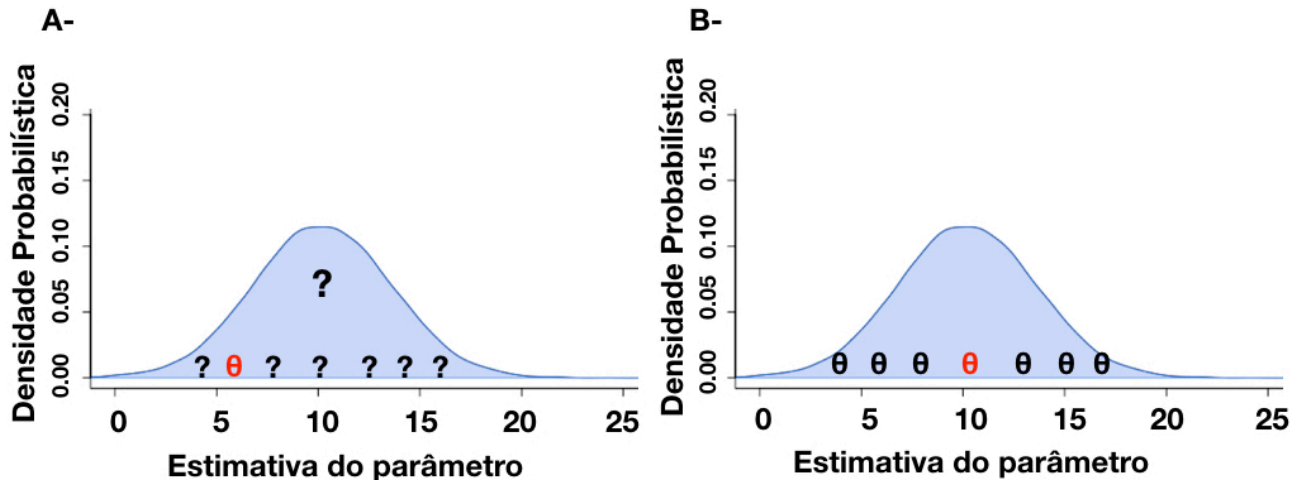


Figura 1: Comparação entre a interpretação de probabilidade nos métodos Frequentista (A) e Bayesiano (B). A análise Frequentista (A) considera θ como parte de um conjunto de infinitas estimativas de parâmetros que compõe uma distribuição de probabilidade ou populacional. No entanto, as demais estimativas de parâmetros (?) do conjunto e a distribuição de probabilidade são desconhecidos. A análise Bayesiana (B) mede a incerteza das estimativas de parâmetros que compõe a distribuição de probabilidade com base nos dados obtidos pelo experimento (θ). Portanto, as estimativas dos parâmetros e a distribuição de probabilidade são conhecidos e levados em consideração na inferência.

Os dados de interações agonísticas são usualmente analisados utilizando a distribuição normal. Contudo, esses dados também podem ser vistos como um processo de Poisson, pois são compostos por dados discretos (Ventura et al. 2015, Sadoul et al. 2016, Slavík et al. 2016). Além disso, métodos de reamostragem possibilitam simular dados, aumentando o número de amostras, ajudando na aproximação com a distribuição desejada (e.g. bootstrap paramétrico, estimativa por máxima verossimilhança) (Davison 1997, Paik et al. 2015). No entanto, ajustes mais fáceis e exatos para modelos mais complexos são obtidos nas análises Bayesianas usando as cadeias de Monte Carlo Markov (Clark 2005, Kinas & Andrade 2010). Esse método lida melhor com situações onde o

número de réplicas é menor, o que possibilita resultados mais robustos do que os Frequentistas (Zellner 1971, 1998).

Existem softwares capazes de fazer análise Bayesiana, alguns deles requerem conhecimento de programação (por exemplo, software R - R Development Core Team 2016; Python e; Stan - Carpenter et al. 2017), outros não (por exemplo, Jasp - Marsman & Wagenmakers 2016). Os softwares de programação têm a vantagem de serem mais plásticos com pacotes estatísticos específicos que atendem às necessidades exigidas por diferentes campos científicos. No entanto, nem todos os pesquisadores estão familiarizados com a linguagem de programação. Recentemente, o pacote de software R chamado Shiny (<http://www.rstudio.com/shiny/>) tornou possível criar ferramentas estatísticas online usando os pacotes estatísticos do software R. Ainda não existem softwares projetados especificamente para explorar dados longitudinais no contexto de estudos de comportamento agressivo.

Considerando a importância dos estudos de comportamento agressivo para o bem-estar dos peixes na aquicultura, o conhecimento sobre delineamentos longitudinais é crucial para a compreensão adequada da agressão dos peixes. Este trabalho tem como objetivos: (i) Testar se uma abordagem Bayesiana aprimora a análise de modelos longitudinais em estudo com comportamento agressivo dos peixes. Foram discutidas as vantagens da análise Bayesiana ao lidar com dados totalmente combinados, resultados estatisticamente não significativos e número de réplicas utilizando o Acará Bandeira (*Pterophyllum scalare*) como estudo de caso; (ii) Investigar se agregar variáveis, utilizando como critério o custo energético dos eventos agonísticos, pode causar viés na análise de dados de 4 espécies de ciclídeo. Além disso, verificar qual tipo de distribuição (Poisson ou Gaussiana) e o número de réplicas é mais apropriado para cada uma dessas espécies; (iii) Construir uma ferramenta estatística utilizando o pacote Shiny para permitir que modelos Bayesianos se tornem mais acessíveis para estudos longitudinais com agressividade.

APRESENTAÇÃO DO TRABALHO

A tese esta dividida em três capítulos redigidos em inglês sob a forma de artigos científicos. No primeiro artigo os dados de comportamento agressivo são, pela primeira vez, explorados sob o prisma Bayesiano no qual foram discutidos métodos e aplicações da análise para dados de comportamento agressivo. Este artigo foi publicado na revista *Behavioral Processes* (ANEXO) e está apresentado de acordo com as normas da revista. No segundo artigo verificamos se existe viés da combinação de variáveis utilizando como critério o custo energético dos eventos agonísticos de 4 espécies de uma mesma família (Cichlidae). Foram escolhidas espécies com diferentes perfis agonísticos para aumentar os contextos nos quais combinar variáveis pode ou não gerar viés. Além disso, foram utilizadas as variáveis parcialmente combinadas e individuais, pois essa classificação apresenta um número menor de variáveis agrupadas facilitando a análise de dados. No terceiro artigo apresentamos a ferramenta online *BayesBehav*, cujo objetivo é facilitar a utilização dos métodos Bayesianos nos dados de comportamento agressivo. Este artigo vem acompanhado de um manual como material suplementar disponível na página 104 deste manuscrito. O segundo e terceiro artigos serão submetidos em breve e estão apresentados de acordo com as normas das revistas *Behavioral Processes* e *Methods in Ecology and Evolution*, respectivamente.

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CAPÍTULO II

BAYESIAN ANALYSIS IMPROVES EXPERIMENTAL STUDIES ABOUT TEMPORAL PATTERNING OF AGGRESSION IN FISH.

Bayesian analysis improves experimental studies about temporal patterning of aggression in fish.

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Ethical Note

This study was carried out in accordance with the ethical principles of the National Council for the Control of Animal Experimentation (CONCEA, Brazil) and was approved by the Ethics Committee for Animal Experimentation of the São José do Rio Preto campus of São Paulo State University, known locally as the Universidade Estadual Paulista (UNESP), in Brazil (permit number 077/2013). All fish handling was preceded by anesthesia, and substantial efforts were made to minimize animal suffering, as recommended by the Animal Behavior Society's Guidelines.

Running head: Bayesian analysis improves studies about aggression in fish.

Abstract

This study aims to describe a Bayesian Hierarchical Linear Model (HLM) approach for longitudinal designs in fish's experimental aggressive behavior studies as an alternative to classical methods. In particular, we discuss the advantages of Bayesian analysis in dealing with combined variables, non-statistically significant results and required sample size using an experiment of angelfish (*Pterophyllum scalare*) species as case study. Groups of 3 individuals were subjected to daily observations recorded for 10 minutes during 5 days. The frequencies of attacks, displays and the total attacks (attacks + displays) of each record were modeled using Monte Carlo Markov chains. In addition, a Bayesian HLM was performed for measuring the rate of increase/decrease of the aggressive behavior during the time and to assess the probability of difference among days. Results highlighted that using the combined variable of total attacks could lead to biased conclusions as displays and attacks showed an opposite pattern in the experiment. Moreover, depending of the study, this difference in pattern can happen more clearly or more subtly. Subtle changes cannot be detected when p-values are implemented. On the contrary, Bayesian methods provide a clear description of the changes even when patterns are subtle. Additionally, results showed that the number of replicates (15 or 11) invariant the study conclusions as well that using a small sample size could be more evident within the overlapping days, that includes the social rank stability. Therefore, Bayesian analysis seems to be a richer and an adequate statistical approach for fish's aggressive behavior longitudinal designs.

Key words: Aggressive behavior, Bayesian analysis, Longitudinal design.

1 Introduction

In aquaculture, several species of fish are social and interact aggressively to set a dominance hierarchy and for territorial defense. Aggressive behavior can be showed in a very dynamic way, escalating in minutes, hours and days. These dynamic processes can cause injuries and, if continued for long time, lead to individual death, or to increased social stress that, in turn, cause negative effect on growth and general welfare (Damsgård & Huntingford 2012). Hence, knowledge about the temporal dynamic of aggressive behavior can benefits fish's management, enhancing species growth and welfare (Dow 1976, Gonçalves-de-Freitas et al. 2008, Damsgård & Huntingford 2012, Montero et al. 2009). Indeed, bibliography of studies intending to evaluate aggressive behavior in fish is increasingly growing (Gómez-Laplaza & Morgan 1993, Ros et al., 2006, Amazán-Rueda et al. 2003, Castro & Caballero 2004, Balzarini et al. 2014). In these articles, ethograms are usually used to understand how fish aggressively interact and to standardize variables patterns for quantifying the number of aggressive responses. Some typical examples of these variables are mouth attacks, lateral threats, and tail beating that can be used individually, partially combined based on its intensity (e.g. attacks and displays), or fully combined in a single one (e.g. total attacks) (Hallen & Wittenberger 1987, Gomez-Laplaza & Morgan 1993, Werneyer et al. 2002, Desjardins et al. 2012, Balzarini et al. 2014). Combining variables have become a common practice among fish aggressive behavior studies (Gonçalves-de-Freitas et al. 2008, Maan et al. 2001, Ros et al., 2006, Amazán-Rueda et al. 2004, Castro & Caballero 2004), although there is still no clear the mathematical criteria about when and how do it.

However, several quantitative problems arise when aggressive behavior data are analyzed using both individual and combined variables, because the collection of this information usually presents three main issues: (i) replicates are hard to produce; (ii) the way which these behaviors occur is quite variable within individuals and; (iii) the experimental design is longitudinal (i.e. concerns the same individual over time). The use of classical approaches to account these issues could be problematic as it imposes difficulties in respecting classical parametrical assumptions as

normality, homoskedasticity, and sphericity. Additionally, multiple comparisons are usually performed using post-hoc tests (e.g. Telegraph 2004, Carvalho et al. 2012). Nevertheless, when multiple comparisons are implemented, the probability that a researcher wrongly conclude that there is at least one statistically significant effect across a set of tests, increases additively with each test (Gelman 2012). In addition, the evaluation of repeated measures when there are more than one treatment/independent variables and the data are non-parametrical is still a limitation in classical approaches.

The Repeated Measures Analysis of Variance (RM ANOVA) is often used in fish's aggressive behavior studies (e.g. Gómez-Laplaza & Morgan 1993, Ros et al., 2006, Amazán-Rueda et al. 2003, Castro & Caballero 2004). Alternatively, the use of Bayesian Hierarchical Linear Models (Bayesian HLM) is grown in popularity highlighting its usefulness in many biological fields including behavioral studies (Alterovitz et al. 2007, McCarthy 2007, Kinas & Andrade 2007, McNamara & Olsson 2006). Bayesian HLMs operate in a multilevel way making comparisons among groups and examining the posterior distributions from different perspectives or margins (Kruschke 2015). Hence, they are more suitable for dealing with multiple comparison problems, because they shift point estimates and their corresponding intervals closer to each other instead of making confidence intervals wider as in classical estimates corrections (Gelman 2012). Additionally, Bayesian HLMs provide a different view of the probability, defining it as a metric of uncertainty. On the contrary, the classical view of the probability is defined as the limit of the relative frequency of observed data in an experiment if the same procedure of data collection and analysis was implemented multiple times (McCarthy 2007). For this reason, in a classical approach, we are not able to provide the exact probability of difference between two or more data groups as Bayesian methods do. Classical methods use p-values to access the probability of having an extreme result when the same experiment is repeated multiple times. Many researchers have pointed the limitations of using p-values in these cases as this approach ignores important non-

significant results in the conclusions of the studies (Gelman 2013, Ludwig 2005, Pitak-Arnnop 2010).

Bayesian analysis can work with resampling procedures, such as Monte Carlo Markov chains, which can numerically approximate Gaussian or different probability distributions (e.g. binomial, Poisson, etc) (Kruschke 2012). Moreover, Bayesian methods can also implement heteroskedastic robust models that can overcome the assumption of homoskedasticity required in RM ANOVA (Kruschke 2012), even with small samples sizes (Good & Zellner 1971, Zellner 1998). Indeed, as mentioned before, replicates in aggressive behavior studies can be difficult and expensive to be acquired. Consequently, the possibility to reduce sample size without changing the robustness of the conclusions could help scientists dealing with this typical issue.

Considering the importance of aggressive behavior studies for fish's welfare in aquaculture, knowledge on the longitudinal designs of the aggressive behavior is crucial for the proper understanding of aggression in fishes, and its consequences for fish social group as well. Within this context, for the first time, we discuss the advantages of Bayesian analysis in dealing with gathered data, non-statistically significant results and required sample size. Therefore, this paper aims to test if a Bayesian HLM approach improves modeling longitudinal designs in aggressive behavior study in angelfishes, a social rank organized cichlid fish (e.g. Carvalho et al., 2012).

2 Materials and Methods

2.1 Subjects and Maintenance

The subject species was the angelfish, *Pterophyllum scalare* Schultze 1823. This fresh water cichlid presents a well known agonistic interaction and social rank organization (Gomez-Laplaza & Morgan 1993). The animals were provided by the Aquaculture Center (CAUNESP) of the São Paulo State University (UNESP) from Brazil. Individuals were kept for 15 days in water tanks of 500L polyethylene (ca. 1 fish/10L), and fed with a commercial ration developed for cichlids, twice a day, in the morning and evening (8:00 and 18:00), until apparent satiety. The water temperature was

controlled to 28° C (Perez et al. 2003) and its quality was maintained through biological filters (filter 400L/h) and constant aeration. The photophase was kept on 12 hours of light (07:00h to 19:00h). The tanks were siphoned weekly to remove the remnants of food and feces.

2.2 Experimental design

Fifteen groups consisting of three animals with size between 30 and 50 mm (standard length) were kept for 5 days to behavioral observation. In order to confirm consistency, this experiment was repeated twice. Aggressive behavior was daily recorded during 10 minutes. The records were run always between 14:00h and 18:00h to avoid influence of the circadian rhythm. This interval of time was chosen to avoid the influence of competition for food (Gómez-Laplaza & Morgan 2003, Grobler & Wood 2013). The aggressive interactions were quantified by the frequency of aggressive behavior, based on *P. scalare* ethogram (Carvalho et al. 2012). Data was labeled as attacks (more intensive, with contact), displays (less intensive, no contact), and total attacks (attacks + displays) for the analysis (Haller & Wittemberger 1988, Alvarenga & Volpato 1995). Body bites, mouth fights and chasing ere considered as attacks, while lateral threat, frontal displays and ripples as displays.

2.3 Statistical Modeling

Modeling was done with data of attacks, displays and total attacks of each trial that were adjusted with Monte Carlo Markov chains (Metropolis-Hasting algorithm) using non-informative priors (Jeffreys 1961).

2.3.1 Modeling the rate of increase/decrease

Bayesian regression analysis was performed for measuring the rate of increase/decrease of the aggressive behavior per day (agB/day). The model was specified as:

$$W_i \sim N(\mu_i, \tau_i)$$

$$\mu_i = \alpha + bY_i$$

$$\alpha \sim N(\vartheta, t) \tag{2.1}$$

$$b \sim N(0, 0.10E-6)$$

$$\vartheta \sim N(0, 0.10E-6)$$

$$t \sim \text{Gamma}(0.001, 0.001)$$

where W_i indicates the error term for i observations with a normal distribution, σ and t are hyperparameters that specify random variances for the intercept α and slope b of the linear model. Uninformative priors were assigned for all the parameters.

2.3.2 Modeling the probability of difference

A Bayesian HLM was performed to access the probability of difference among days using the last day as the reference class (Weltezel et al. 2012). Subsequently, it was calculated the probability that treatments are equal, *i.e.* their difference is equal to 0. For this purpose, it was used a boolean variable that counts the number of simulations in which the difference among days are ≥ 0 or < 0 , representing a Bayesian analogue to the classical approach p-value (Gelman et al. 1996, Kéry & Royle 2016). In this case, the model was specified as:

$$W_i \sim N(\mu_i, \tau [Y_i])$$

$$\mu_i = \alpha + b[Y_i] \tag{2.2}$$

$$b \sim N(\vartheta, t)$$

$$\alpha \sim N(0, 0.10E-6)$$

$$\vartheta \sim N(0, 0.10E-6)$$

$$t \sim \text{Gamma}(0.001, 0.001)$$

$$\tau \sim \text{Gamma}(0.001, 0.001)$$

where W_i indicates the error term for i observations with a normal distribution and b specifies the average for each category Y for the parameter μ . The hyperparameters ϑ and t indicate random

variances for the parameter b and α parameter refers to the intercept (reference class) and is specified by an uninformative prior distribution. As in the precedent model, uninformative priors were assigned for all the parameters.

2.3.3 Reducing sample size

For measuring the feasibility of reducing the sample size, we performed a simulation study, using our data. We used the same model of the probability differences described above. However, we performed it multiple times, randomly taking one replicate away from the data each time. The Cohen d effect size (1988) was calculated to measure the impact of reducing the sample size in data. It was considered a value of 0.2, 0.5 and 0.8 for the small, medium and large effects, respectively (Cohen 1988).

2.3.4 Plotting the results

A Bayesian model without any reference class parameters was adopted for plotting the results graphically. All the posterior distributions were plotted in parts for didactic purposes.

Here, the model was specified as:

$$\begin{aligned}
 W_i &\sim N(\mu_i, \tau[Y_i]) \\
 \mu_i &= b[Y_i] \\
 b &\sim N(\vartheta, t) \\
 \vartheta &\sim N(0, 0.10\text{E-}6) \\
 t &\sim \text{Gamma}(0.001, 0.001)
 \end{aligned} \tag{2.3}$$

where W_i indicates the error term for i observations with a normal distribution and b indicates the average for each category Y for the parameter μ . The hyperparameters ϑ and t specify random variances for the parameter b . Uninformative priors were assigned for all the parameters.

2.4 Software and Simulations

Bayesian models were computed using the JAGS software (Just Another Gibbs Sampler) and statistical package “*rjags*” (Plummer et al. 2006), specifically developed for implement Bayesian methods using the Monte Carlo Markov chains through the R software (R Development Core Team 2016). The treatments parameters were estimated through simulations. The number of simulations was established by trial and error in the model convergence process. The success or failure in convergence was checked using the criteria of Geweke (Geweke 1992) and Gelman-Rubin (Gelman & Rubin 1992). The latter was also used for establishing a period of “*burn-in*” constituted by the disposal of some chains to help the model convergence.

3 Results

3.1 Rate of increase/decrease and difference probability among days in fish’s attacks and displays

For the first trial, the displays increased till the third day at a rate of 21 ± 3 agB/day. This increase was very consistent since the posterior distributions from the first to the third day have not overlapped, being a 100% different from each other. At the fourth day, displays still increased, although this rate dropped to 17.9 ± 1.8 agB/day (Figure 1 A-1). This drop caused an overlap between the distribution of the fourth and third day, differing in 91% from each other. At the fifth day, the agB remarkably decreased with a 98% difference from the fourth day (Figure 1 A-2). The attacks increased until the second day (not enough data to calculate a rate) (Figure 1 B-1) and retreated from the second to the fifth day at a rate of -4.1 ± 1.5 agB/day (Figure 1 B-2). Different from displays, there was a high overlapping of the distributions from the second to the fourth day and an increase in difference at the fifth day. It is noteworthy that the attacks and displays were almost the same till the second day, differing in 83% (Figure 2). However, at the third day, both behaviors became completely non overlapped till the rest of the study period. In addition, the credible interval for the standard deviation among days more than encompassed the standard

deviations within each day (Table 1, Figure 3 A-1 and A-2), meaning that there is more uncertainty in differences among days than within each day for this trial.

For the second trial, the displays increased till the third day at a rate of 27.9 ± 3.72 agB/day (Figure 4 A-1). The second and third day overlapped in 8%. The displays decreased till the fifth day at a rate of -2.5 ± 5 agB/day (Figure 4 A-2). Although displays decreased at the fourth day, this day was very similar to the third and the fifth ones, differing only in 71% and 54%, respectively. The attacks increased from the first to the second day (not enough data to calculate a rate), decreasing till the fifth day at a rate of -8.6 ± 1.7 agB/day (Figure 4 B-1 and B-2). Different from the first trial, there was a high overlapping only from the second to the third day. At the fourth and fifth day, the decrease was clearer with a slightly overlapping of the distributions on the fourth day and a not overlapping at the fifth day. As in the first trial, the attacks and displays were the same at the first day ($\neq 50\%$) (Figure 5). Although, at the second trial displays and attacks were more likely to differ on the second day ($\neq 94\%$, compared to 83% in the first trial). Additionally, the rate of increase of displays (till the third day) and a decrease of attacks differed in 92% and 96% between trials, respectively. In addition, the credible interval for the standard deviation among days more than encompassed the standard deviations within each day, in most cases (Table 1, Figure 3 B-1 and B-2). The only exception was the second day of the variable attacks. Therefore, in most cases, there was more uncertainty in differences among days than within each day for this trial.

3.2 Rate of increase/decrease and difference probability among days in fish's total attacks

Overall, the displays were much higher than the attacks. Therefore, they prevailed on the total attacks. Since, attacks retreated and displays increased after the second day, gathering these variables, negatively impacted the rate of increase reducing it from 18 ± 1.8 agB/day to 17.3 ± 3 agB/day at the first trial (Figures 6 A-1 and Figure 1) and from 27.8 ± 3.6 agB/day to 24 ± 8 agB/day at the second (Figure 6 B-1 and Figure 4). This rate reduction is due to the overlapping of the distributions from the days after the second one, that is the period in which attacks and displays followed different paths (Figures 2 and 5). Hence, the difference between the second and third day, which is a

100% of displays, dropped to an 84% of total attacks in the first trial. At the second trial the difference of 92% at displays dropped to 79% at total attacks. The rate of increase between total attacks trials differed in 84%.

3.3 Reducing sample size

For displays of the first trials, the reduction of the data sample size tended to decrease the posterior probability of difference among all the days. The unique exception was found between the third and fourth day, in which the posterior probability did not present a clear pattern, also showing a small effect size (Table 2). The difference among the first three days remained the same till the tenth replicate ($\neq 100\%$), and decreasing till the rest of the sample reduction (Figure 7 A-1). Additionally the effect size between these days was considered large (Table 2). For the attacks, the reduction of the data sample size tended to decrease the posterior probability of difference among the first and second day, and the fourth and fifth day. Between these days there was as medium effect size. From the second till the fourth day there was no clear pattern, with a small effect size (Figure 7 A-2 and Table 2).

At the second trial, for displays, the sample size data reduction tended to decrease the posterior probability of difference among the first three days (Figure 7 B-1). Although, different from the first trial, only the difference among the first and second day remained the same till the seventh replicate ($\neq 100\%$). Between the first and second day there was a very large effect size, while among the second and third days there was a medium effect. For attacks reducing sample size provided similar patterns to the first trial (Figure 7 B-2), although, the first and last two days presented a large effect size instead of a medium one.

4 Discussion

In this study we described how Bayesian analysis could be a reliable alternative to classical methods for longitudinal designs in fish's experimental aggressive behavior studies. Particularly, we

presented the advantages of Bayesian analysis in dealing with combined variables, non-statistically significant results and required sample size using as case study an experiment of angelfish (*Pterophyllum scalare*) species.

Our findings revealed how probability degrees, in accordance with the density distributions, provided a clear description of the changes even when patterns are subtle, which cannot be detected using p-values. In addition, Bayesian analysis is enabled to identify whether it is feasible or not to combine variables and reduce sample size. All these subjects are discussed in the topics below.

4.1 Behavioral consistency

A consistent pattern shows that a specific behavior does not occur by chance. The same experiment, repeated twice, shows a similar pattern for attacks, displays, and total attacks. Gómez-Laplaza & Morgan (1993) have also found similar patterns for *P. scalare*, which reinforce our results. The increase rate of total attacks, attacks, and displays, between the trials, was also similar (differed in 84%, 94%, and 92%, respectively) with the mentioned study. The uncertainty of 8 and 3 agB/day for total attacks, 3 and 3.72 agB/day for displays, and 1.5 and 1.8 agB/day for attacks was small. We can conclude that the rate of increase is almost similar within the replicates of each trial. In addition, the uncertainty of the standard deviation among days was bigger than within days, meaning that most differences are due to time rather than the unknown factors. It is noteworthy, that the second day presented the larger variation within days, for the variable attacks. For the second trial, the variation within this day was even higher than between days. The second day is related to the establishment of the dominance and subordinative relationships within groups, which is related to the increase in high-intensity agonistic behaviors (Ros et al., 2006). There are many known factors which can increase the variability of this social hierarchy, such as size, experience and physiological status (Damsgård & Huntingford 2012). Hence, this high variation within the second day is probably related to the social rank stability.

4.2 Statistical significance

Aggressive behaviors are subject to changes during the time (Dow 1976). However, these changes can happen so subtly that may not be statistically noticed in a short period of time. For example, in total attacks the second day is 83% different from third and 95% different from day fourth, but the third day is 77% different from the fourth day (Figure 6 A-1). Using uninformative priors, Bayesian analysis may provide results similar to classical approaches (Jaynes 1976). When we find 95% of difference, probably we also would find significance with the classical approach ($p < 0.05$). In this case, between days 2 and 3 and 3 and 4 there was no statistical significance considering a 95% credible interval. On the contrary, a statistical difference was found between days 2 and 4. Usually, when this happens, researchers state that the aggressive behavior is the same between the non-statistically significant days even when there is an increase/decrease pattern (e.g. Gómez-Laplaza & Morgan 1993, Adams et al. 2000, Terleph 2004, Gonçalves de Freitas & Mariguela 2006). Indeed when considering p-values we might misinterpret non-significant results (Gelman 2013, Ludwig 2005, Pitak-Arnnop 2010). Bayesian analysis clarifies the longitudinal changes by building the agB distribution in each day and calculating the probability of difference between them. Therefore, we can measure the strength of the evidence against the null hypothesis (days are equal) using probability degrees (Wagenmakers 2007). A very intuitive classification was proposed by Raftery (1995) where the categories 50% to 75%, 75% to 95%, 95% to 99% and >99% indicates weak, positive, strong and very strong evidence against the null hypothesis, respectively. At the total attacks example, the probability that the third day differs from the second is 83%, and it increases to 95% on the fourth day. Hence, the third day is a transition from second day to fourth day, going from a positive to strong evidence against the null hypothesis. Therefore, the Bayesian approach provides an idea of changes and continuity among days even with the lack of statistical significance, which is more intuitive for a longitudinal design than the equality idea provided by classical methods.

4.3 Combining variables

As the numbers of less intensive aggressive behaviors (displays) were much higher than the numbers of the more intensive ones (attacks), they prevailed on the total attacks, shaping its distribution through time. In the literature, there are many articles using total attacks (e.g. Amazán-Rueda et al. 2003, Wessel et al. 2004, Castro & Caballero 2004, Early 2006, Miyai et al. 2011, Carvalho et al. 2012, Pinho-neto et al. 2014). However, our results highlight that adding attacks to displays diminishes the posterior probability between the days compared to using displays alone, losing their statistical significance considering a 95% credible interval (Figures 1 A-2 and 5 A-1). This is because attacks and displays are different things in fish's social environment. Attacks (which require more energy) retreat in time when dominance hierarchy is established and displays (requires less energetic cost) increases (Haller & Wittenberger 1988, Maan et al. 2001, Ros et al., 2006).

This pattern is also described for other species such as juvenile *Oreochromis niloticus* (Alvarenga & Volpato 1995), *Tilapia zilli* (Neat et al. 1998) and *Oreochromis massambicus* (Ros et al., 2006). Although, this pattern is not clear for all species, as in hatchery-reared steelhead (*Oncorhynchus mykiss*) (Berejikian et al. 2001) and oscar (*Astronotus ocellatus*) (Gonçalves-de-Freitas & Mariguela 2006). In this sense, whether or not using total attacks may be species-specific and should be previously investigated. In this context, Bayesian analysis can assess the impacts of combining data as attacks and displays by exhibiting the changes in probabilities that happens when using a variable alone and combined. Researchers could use the Bayesian tool to analyze the impact of combining the same variables for other species or other aggressive variables such as bites, chasing or tail beats. Considering the Raftery (1995) rule of thumb any 5% of difference at the posterior probability against the null hypothesis is enough to change from strong (95% to 99%) to positive evidence (75% to 95%). Therefore, we suggest a 5% change in the posterior distribution as a criterion to not combining data. It is noteworthy that the best procedure will be always to compare the combined variable with the more quantitative single variable (in our example displays).

Consequently, the expected effect is always negative or nonexistent since the predominant variable is the main one at the posterior distribution of the gathered variable. The same issue could be differently investigated with classical approaches checking if p-values are >0.05 or <0.05 when using the data gathered or not. However, even using this approach the amount of probability change would not be measured. Indeed, if for example, with gathered data the p-value will be 0.01 and with not gathered data of 0.06, this does not mean that using gathered data decreases in 5% the data difference (that is what Bayesian analysis can conclude). On the contrary, it decreases by 5% the probability of having an extreme result if the same experiment is repeated multiple times. Therefore, classical approaches did not measure the impact of combining in our experiment, but the effect of repeating it. Additionally, since classical approaches are mainly focused on p-values and statistical significance, it would not be applied to non-statistically significant results and these can be important in aggressive behavior longitudinal studies as explained in the topic above.

4.4 Reducing sample numbers

The impact of using a small sample size was more evident in days that overlapped more. The inference among these days presented smaller effect sizes. When the effect size of the intervention is large, it is possible to detect such effect in smaller sample numbers, whereas a smaller effect size would require larger sample size (Sullivan & Feinn 2012). Therefore, attacks were more sensitive to sample numbers from the second till the fourth days, given the smaller effect size and high overlapping among agB distribution. The displays were more sensitive among the third and the fourth day, which are the climax of increase for the first and second trials, respectively. Therefore this sensitivity is due to the uncertainty of the display's increase climax, which can happen at the third or fourth day depending on the individual. Besides the increase in variability, the expected pattern of increase of the agB for displays and decrease for attacks remained robust till the eleventh and tenth replicates, at the first and second trials, respectively. This pattern is related to the social rank stability (Alvarenga & Volpato, 1995; Neat *et al.*, 1998; Ros *et al.*, 2006). After the tenth

replicate the variability reaches a level of increase that causes the misleading idea that the agB distribution of some days are equal. Hence, a small sample size could provide the false idea that the agB does not change after dominance hierarchy being established. As previously mentioned, when we rely on p-values with 95% intervals we ignore important no significant information. Thus, the sample size is important to acknowledge the transitional pattern of the agB among days. Therefore a small sample number (≤ 10) can ignore these subtle changes, wrongly transmitting the idea that aggressive behaviors are the same at some days, and even Bayesian estimation can be biased.

The social rank stability is related to this pattern of decrease of the attacks and increase of the displays (Alvarenga & Volpato, 1995; Neat *et al.*, 1998; Ros *et al.*, 2006). Hence a small sample size could provide the false idea that the agB did not change after dominance hierarchy being established.

Usually articles concerning sample size use power rather than effect size. Effect size and power are related. However, power considers a 95% interval and we are interested at differences that are below this interval. Therefore, we have chosen to work only with the effect size, since it fits better to our proposal.

5 Conclusions

For *P. scalare* the use of total attacks could lead to biased conclusions as displays and attacks showed an opposite pattern in the experiment. The daily changes in aggressiveness happen subtly and cannot be detected when p-values are implemented. On the contrary, Bayesian methods provide a clear description of the changes even when patterns are subtle. Additionally, results showed that the number of replicates (15 or 11) invariant the study conclusions as well that using a small sample size could be more evident within the overlapping days, that includes the social rank stability. Therefore, we can conclude that the Bayesian approach could be a richer and an adequate statistical framework for fish's aggressive behavior longitudinal designs.

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Figures

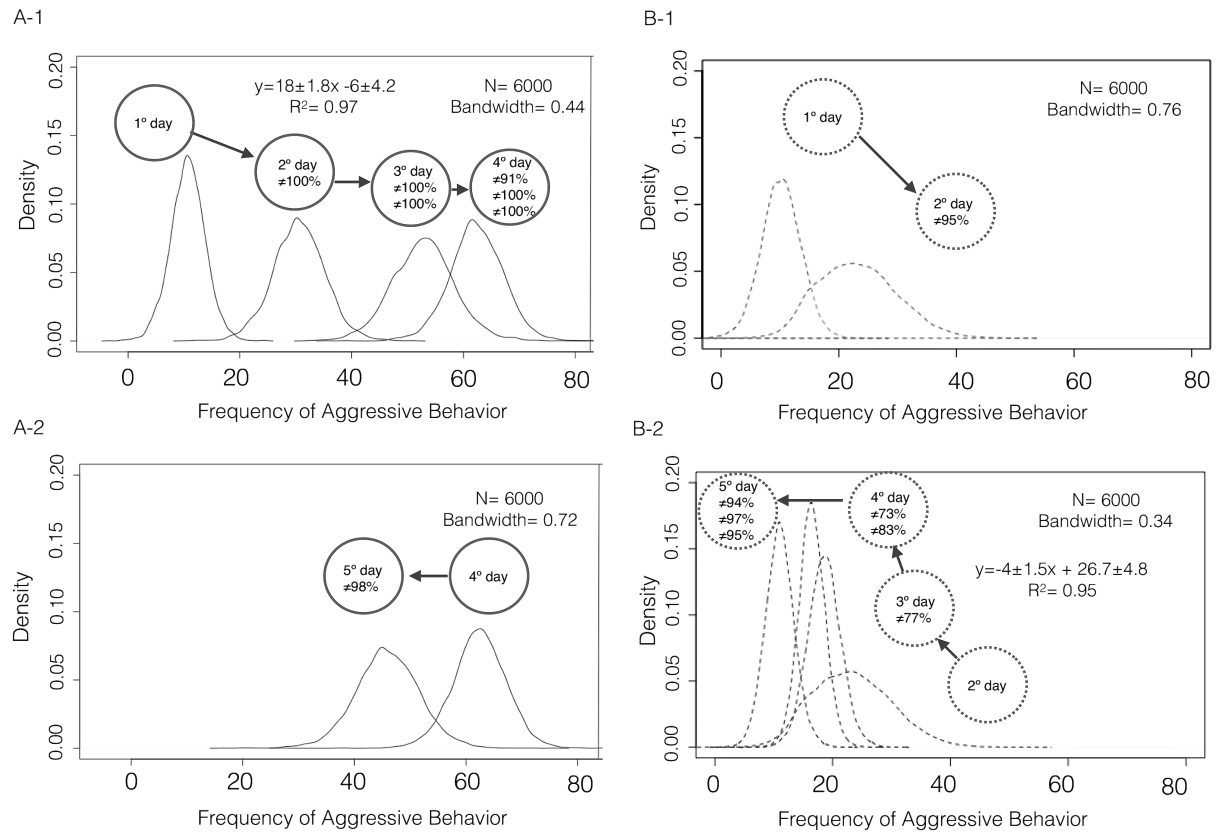


Figure 1: The daily agB density distributions of the displays (A-1 and 2) and attacks (B-1 and 2) of the first trial. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

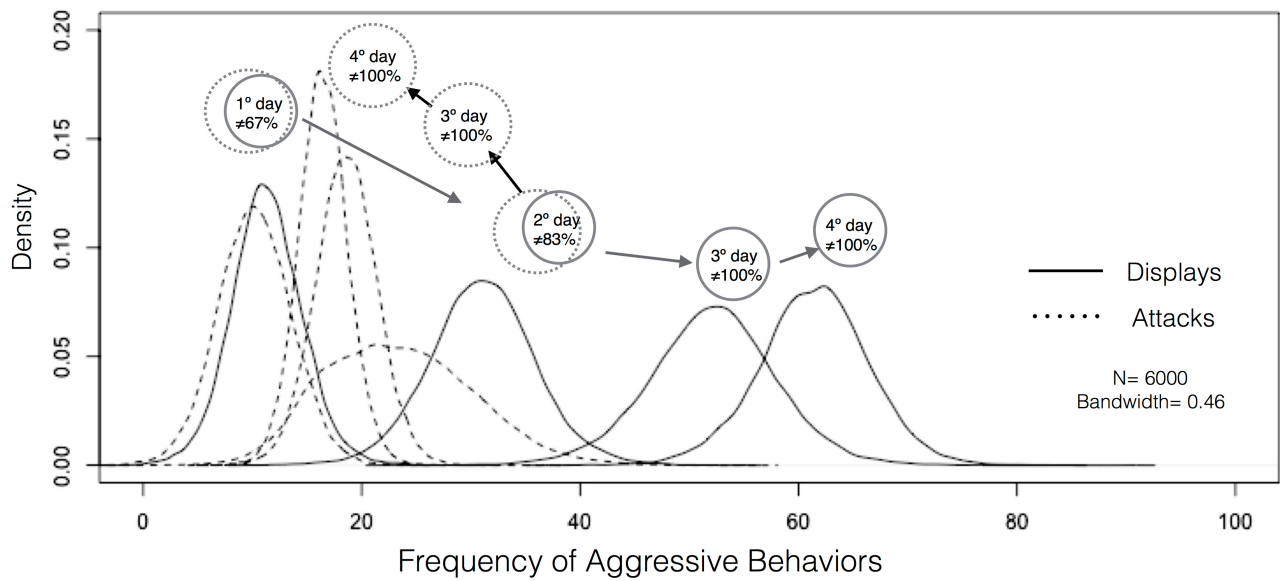


Figure 2: The daily agB density distributions of the attacks and the displays of the first trial. It was observed in 15 minutes at each day. They are represented by solid and dashed lines, respectively. Each circle contains the probability of difference between attacks and displays at each day.

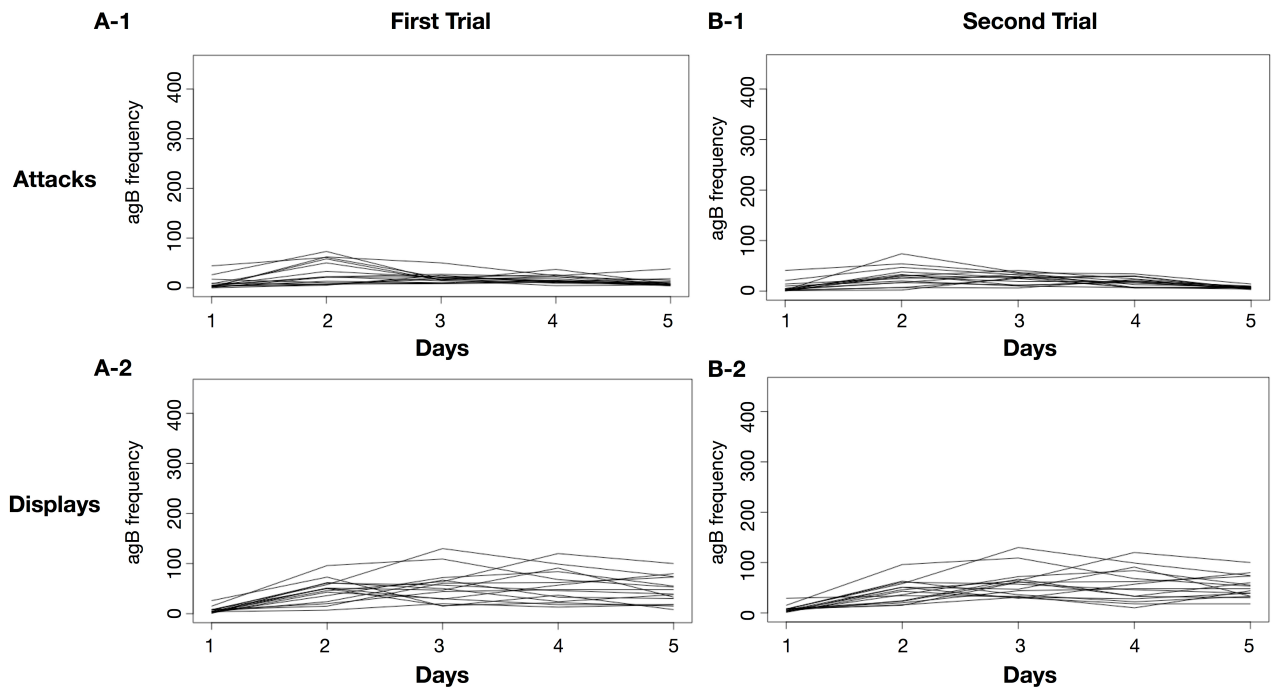


Figure 3: The daily agB frequency of each of the fifteen groups in five days of observation. It was observed in 15 minutes at each day.

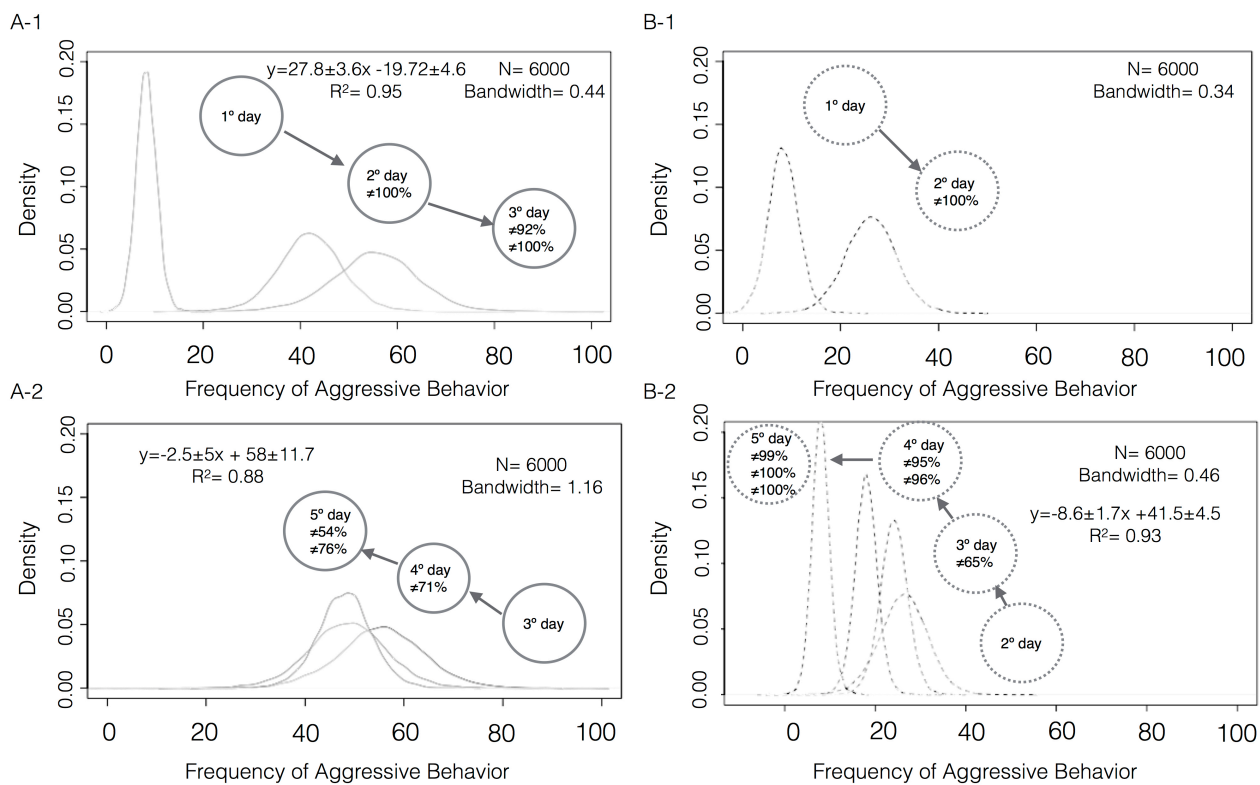


Figure 4: The daily agB density distributions of the displays (A-1 and 2) and the attacks (B-1 and 2) of the second trial. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

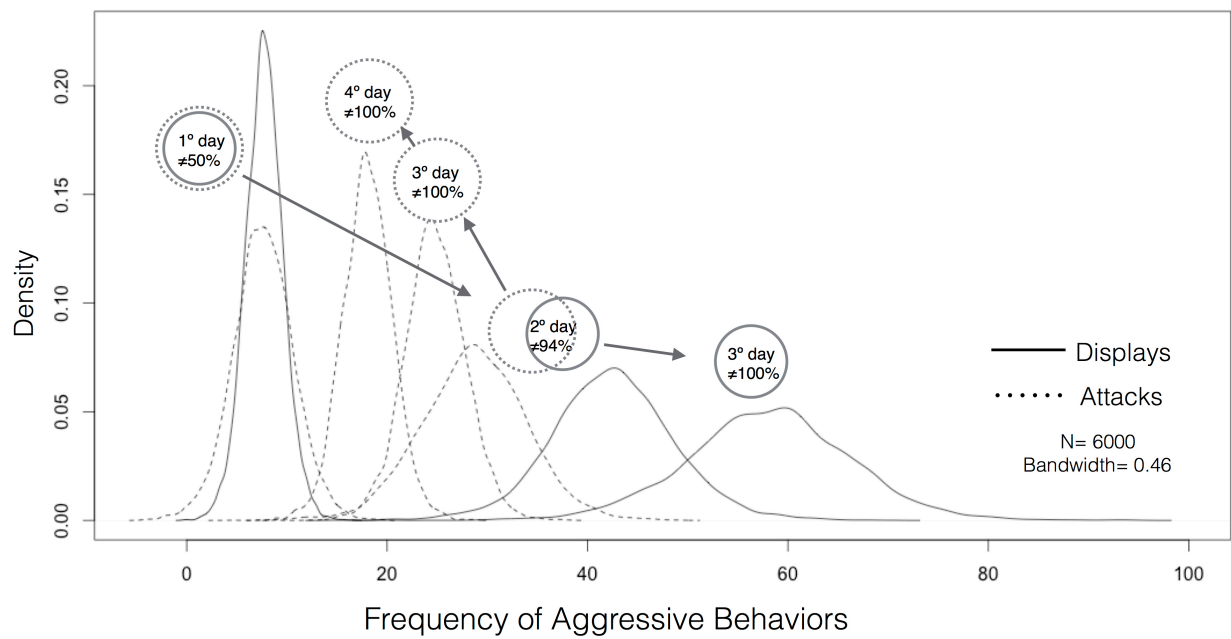


Figure 5: The daily agB density distributions of the attacks and displays of the second trial. It was observed in 15 minutes at each day. They are represented by solid and dashed lines, respectively. Each circle contains the probability of difference between attacks and displays at each day.

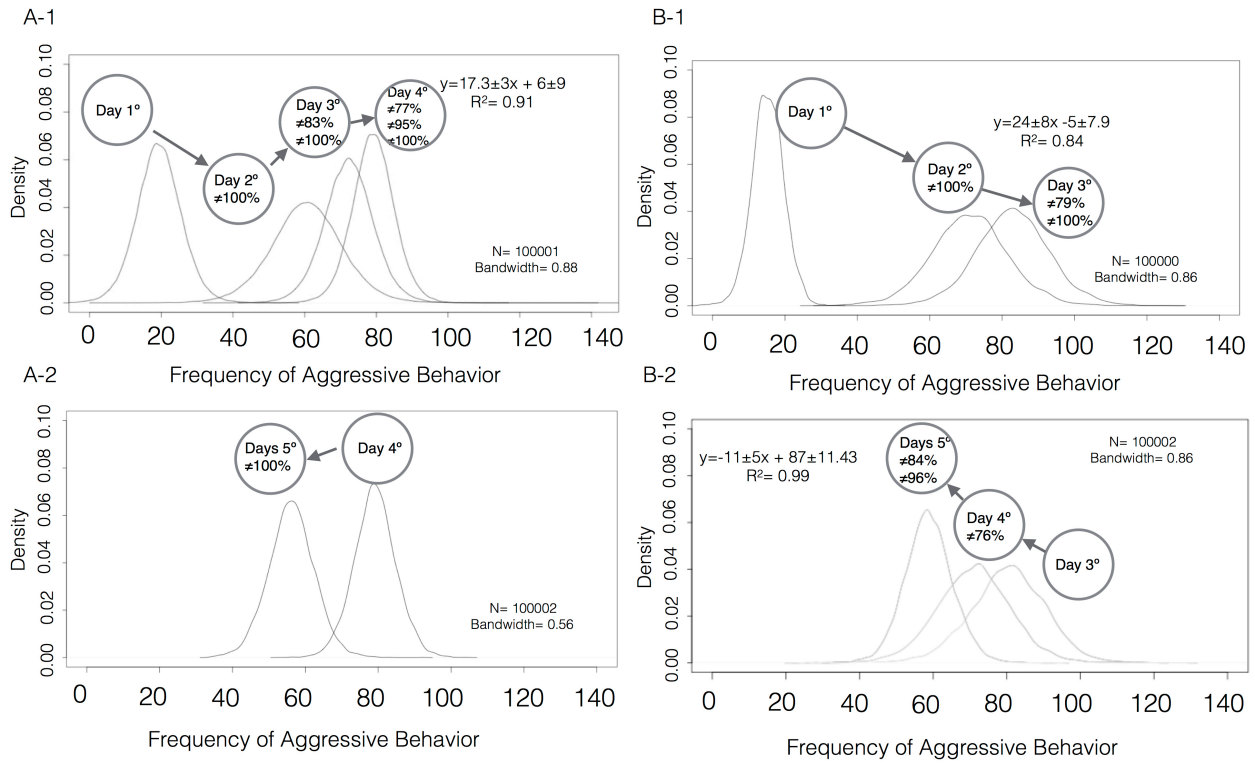


Figure 6: The daily agB density distributions of the total attacks. A-1 and A-2 represents the first trial. B-1 and B-2 represents the second trial. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

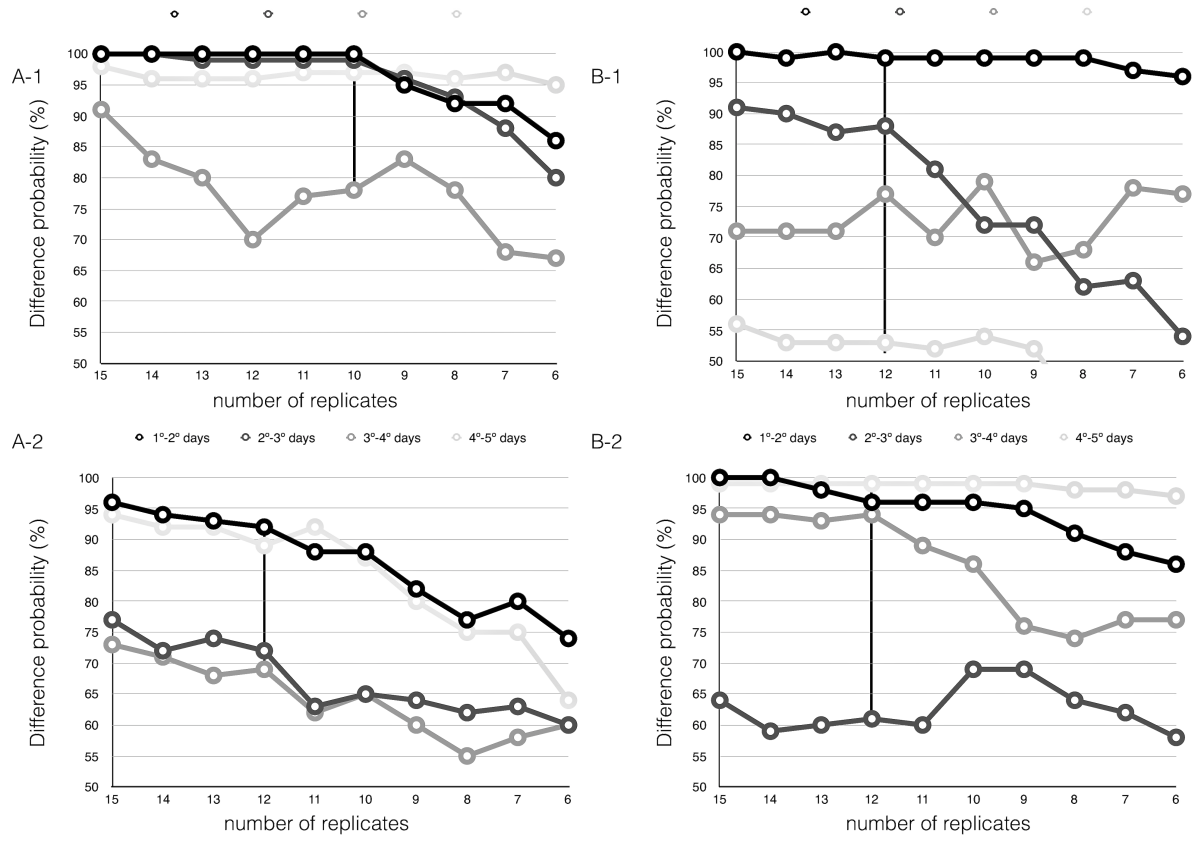


Figure 7: The distribution of difference between days with different sample sizes. A-1 and 2 represents the displays and attacks of the first trial. B-1 and B-2 represents the displays and attacks of the second trial. The black line is between the first and second day, the dark gray line is between the second and third days, the gray is between the third and fourth days and the light is between the fourth and fifth days. Black line is the cut point.

Tables

Table 1: The mean and 95% credible interval for the standard deviation among and within days of the attacks and displays variables in two trials.

	Attacks		Displays	
	Mean	95% CI	Mean	95% CI
First Trial				
Among all days	13.95	5.27-36.95	30.51	13.02-71.81
Within 1° day	12.00	8.15-18.34	7.48	5.05-11.29
Within 2° day	21.26	14.54-32.12	24.20	16.65-36.68
Within 3° day	12.08	8.23-18.53	32.12	21.85-48.67
Within 4° day	9.86	6.76-14.67	35.79	24.37-54.37
Within 5° day	3.07	2.08-4.66	24.47	16.69-37.22
Second Trial				
Among all days	9.49	1.7-28.0	29.85	12.56-72.42
Within 1° day	13.31	9.0-20.2	12.53	8.58-18.92
Within 2° day	27.66	18.6-42.2	18.60	12.74-28.36
Within 3° day	11.20	7.6-17.1	22.34	15.18-34.21
Within 4° day	9.12	6.2-13.8	19.27	13.15-29.24
Within 5° day	9.63	6.5-14.6	22.91	15.56-34.66

Table 2: Effect sizes between days.

<i>Days</i>	<i>Displays First Trial</i>	<i>Attacks First Trial</i>	<i>Displays Second Trial</i>	<i>Attacks Second Trial</i>
1-2	1.27	0.64	2.00	1.00
2-3	1.00	0.23	0.50	0.12
3-4	0.45	0.20	0.10	0.56
4-5	0.77	0.60	0.06	0.90

CAPÍTULO III

THE BIAS OF COMBINING VARIABLES ON FISH'S AGGRESSIVE BEHAVIOR STUDIES

THE BIAS OF COMBINING VARIABLES ON FISH'S AGGRESSIVE BEHAVIOR STUDIES

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Ethical Note

This study was carried out in accordance with the ethical principles of the National Council for the Control of Animal Experimentation (CONCEA, Brazil) and was approved by the Ethics Committee for Animal Experimentation of the São José do Rio Preto campus of São Paulo State University, known locally as the Universidade Estadual Paulista (UNESP), in Brazil (permit number 169/2017). Data from experiments performed in previous studies were also used. These studies were also carried out in accordance with ethical principles from the CONCEA and the Ethics Committee for Animal Experimentation from the UNESP (permit numbers 077/2013; 083/2013). All fish handling was preceded by anesthesia, and substantial efforts were made to minimize animal suffering, as recommended by the Animal Behavior Society's Guidelines.

Running head: The bias of combining variables on fish's aggressive behavior studies.

Abstract

Combining aggressive variables based on its intensity, as displays and attacks, is a common practice in animal behavior studies. However, combining variables can generate a bias in data analysis, especially when the variables have different temporal patterns. This study aims to use Bayesian Hierarchical Linear Models (B-HLMs) to analyze the feasibility of pooling aggressive behavior variables in four cichlids species. In addition, we discuss the feasibility to use partially combined variables, to reduce sample size and to apply different family distributions. The subject species were: the angelfish (*Pterophyllum scalare*), the tiger oscar (*Astronotus ocellatus*), the *Cichlasoma paranaense* and the Nile tilapia (*Oreochromis niloticus*). For each species, 15 groups of 3 individuals were subjected to daily observations (10-min recording) for 5 days. Aggressive behavior data was classified according to its aggressive intensity. The variables chase (C), tail beating (TB), push (P), lateral attack (LA) and bite (B) were classified as high intensity. The variables undulation (U), lateral threat (LT) and frontal displays (FD) were classified as low intensity. These behaviors, however, were not present in all species. Models were adjusted by Monte Carlo Markov chains using non-informative priors. B-HLMs were performed to assess the probability impact of each variable to the analysis. Results highlighted that when combining variables, the resulting distribution is strongly influenced by only one variable in each category. In addition, aggregating values mislead results in some cases, changing the probabilities from the main variables. Species with low frequency in aggressive behavior are more sensitive to this bias. LT was the main low intensity variable for all species, while B was the main high intensity variable for the *P. scalare* and the *O. niloticus*. LA was the variable belonging to the high intensity category with more relevance for the *C. paranaenses* and *A. ocellatus*. Moreover, combining variables had no impact in the feasibility of reducing the sample size compared to using the most quantitative variable. For all species a sample size of 12 invariant the study conclusions. Concerning family distribution, the Gaussian model was more suitable for most of the species, except for the *A. ocellatus*, for which Poisson models fits better than Gaussian one.

Keywords: Aggressive Behavior, Bayesian analysis, Pooled Data

1. Introduction

Aggression is an important part of many social species behaviors and it is used to set up a dominance hierarchy and defending territory (Maan et al. 2001). The aggressive interactions are dynamic and can escalate in different time-ranges depending on the species. However, when prolonged, these interactions can cause bodily injuries and increased social stress, which impair growth and welfare (Damsgård & Huntingford 2012). Because many social species are farmed and fished by the aquaculture and fisheries industry, there are many scientific researches regarding the welfare of cultured and wild fishes.

In these studies the behavioral variables are standardized and described in ethograms (e.g. Galhardo et al. 2008, Carvalho et al. 2012). Some typical examples of these variables are bites, lateral attacks, lateral threats, and undulation. These variables are usually analyzed individually (e.g. Oliveira et al. 2011), partially combined (sum of events based on a given criteria) (e.g. Gomez-Laplaza & Morgan 1993, Desjardins et al. 2012) or fully combined (sum of all aggressive events) (e.g. Gonçalves-de-Freitas et al. 2008, Maan et al. 2001, Ros et al. 2006, Amazán-Rueda et al. 2004, Castro & Caballero 2004). Among these types of classification, the partially combined is the more ambiguous within the literature, because they are displayed with many different names. Some examples are the overt aggression and the restrained aggression (Desjardins et al. 2012, Balzarini et al. 2014), the high and the low intensity attacks (Gomez-Laplaza & Morgan 1993, Werneyer et al. 2002, Gonçalves-de-Freitas & Mariguela 2006), the Asymmetric high-intensity agonistic attacks (AHI) (e.g. Galhardo et al. 2008). Despite the name, these classifications have something in common, they are all based on the energetic costs of the aggressive behaviors (e.g. Gomez-Laplaza & Morgan 1993, Ros et al. 2006, Arnold & Taborsky 2010). Although plausible, no mathematical criteria has been adopted for pooling these variables into a single one. Actually, pooling variables can generate a bias in data analysis, especially when the combined variables are shown in different temporal patterns during fighting events (Dearing et al., 2001, Noleto-Filho et al. 2017).

For aggressive studies, problems in fully combined variables were already acknowledged by Noleto-Filho et al. (2017) for *P. scalare* and it was highlighted that the pattern of the most quantitative behavioral variable usually prevails. In some cases, the other variables (less quantitative ones) cause small deviances on this pattern. Hence, it is likely that the same problem happens to the partially combined variables, since the variables that are partially aggregated share similar properties with the variables fully combined, such as different frequencies and sometimes different patterns (e.g. Gonçalves-de-Freitas & Mariguela 2006, Ros et al. 2006). In addition, these variables are usually analyzed using the Gaussian distribution. However, they can be also analyzed as a Poisson process because it is composed of counts and discrete data (Ventura et al. 2015, Sadoul et al. 2016, Slavic et al. 2016). Noleto-Filho et al. (2017) describe a suitable approach for using Bayesian Hierarchical Linear Models (B-HLMs) and Raftery's criteria (Raftery 1995) to measure the impacts of combining aggressive data with a cichlid species. Their results were reliable, although it was sensitive to the number of replicates (Noleto-Filho et al. 2017). This sensitivity was measured by simulating a sample size reduction and checking if conclusions would remain the same. Therefore, they could identify how many replicates are necessary to have the same conclusion.

The feasibility of partially combined variables is not yet explored for cichlids or any other fish families. Within this family, there are many species with dominance hierarchies being common in one or both sexes and complex social behaviors associated with territoriality and reproduction (Maruska et al. 2014). Also, many of these social species present different aggressive patterns and frequencies (e.g. Carvalho et al. 2012, Gonçalves-de-Freitas & Mariguela 2006, Brandão et al. 2015) which can produce different contexts for data analysis. Therefore, the present study aims to use a B-HLMs to check the feasibility of partially combined variables in four cichlids species. Moreover, a second objective of this study is to verify the suitable number of replicates and the more appropriate family distribution to perform aggressive behavior studies, as these are important issues for models' accuracy.

2. Materials and Methods

2.1. Subjects and Maintenance

The subject species were the *Pterophyllum scalare* (Schultze 1823), the *Astronotus ocellatus* (Agassiz 1831), the *Cichlasoma paranaense* Kullander 1983 and the *Oreochromis niloticus* (Linnaeus 1758). These four freshwater cichlids present aggressive relationships already described in the laboratory (Carvalho et al. 2012, Gonçalves-de-Freitas & Mariguela 2006, Brandão et al. 2015, Carvalho et al. 2008). These species were chosen because of its viability (acquisition), differences in frequency and type of aggressive behavior, that improves our data analysis, providing more contexts in which combining variables could bring or not bias. For the four species, the animals were kept for 15 days in water tanks of 500L polyethylene (ca. 1 fish/10L), and fed with food for cichlids in the morning and the evening (8:00 and 18:00), until apparent satiety. With the exception of tilapia, no distinction regarding sex was made. The water temperature was controlled to 27-28° C (Perez et al. 2003, Kelsch et al. 1990). The water quality was maintained through biological filters (filter 400L/h) and constant aeration. The photoperiod was kept on 12 hours of light (07:00h to 19:00h). The tanks were weekly siphoned to remove the remnants of food and feces.

2.2. Experimental design

For the four species, 15 groups consisting of three animals each were subjected to 5 days of observation in aquaria (40 x 30 x 40 cm; ca. 48 L). Sizes (standard length) between 3-5 cm, 5-10 cm, 6-8 cm and 5-10 cm for the *P. scalare*, *O. niloticus*, *C. paranaenses* and *A. ocellatus*, respectively. Aggressive behavior was daily recorded for 10 minutes. The aggressive interactions were quantified by the frequency of aggressive behavior, based on ethograms described for each species (Table 1) (*P. scalare* and *O. niloticus* - Carvalho et al. 2012; *A. ocellatus* - Gonçalves-de-Freitas & Mariguela 2006; *C. paranaenses* - Brandão et al. 2015). This ethogram is composed of

minor variables which are individual events classified and combined in two major variables: the attacks (more energetic expense, with contact) and the displays (less energetic expense, no contact) (Table 1). This classification was used because it is a common data pooling criteria of most aggressive behavior studies (e.g. Haller & Wittemberger 1988, Gomez-Laplaza & Morgan 1993, Werneyer et al. 2002, Gonçalves-de-Freitas & Mariguela 2006, Arnold & Taborsky 2010).

2.3. Statistical Modeling

The modeling was made with data from each variable that were adjusted with Monte Carlo Markov chains (Metropolis-Hasting algorithm) using non-informative priors (Jeffreys 1961).

2.3.1 Modeling the probability of difference among days

Bayesian Hierarchical Linear Models (B-HLMs) were performed to assess the probability of difference among days (Weltezel et al. 2012), implementing two different family distributions (Gaussian and Poisson). Subsequently, the Bayesian probability of difference was computed, which is a Bayesian index analog to the classical p-value (Gelman et al. 1996, Kéry & Royle 2016). Additionally, the Deviance Information Criterion (DIC) was also calculated as a measure of quality to compare the statistical models. DIC values are inversely related to the compromise between fit and parsimony (Spiegelhalter et al. 2002).

The Gaussian model was specified as:

$$\begin{aligned}
 W_i &\sim N(\theta_i, \tau [Y_i]) \\
 \theta_i &= b[Y_i] \\
 b &\sim N(\mu, t) \\
 \mu &\sim N(0, 0.10E-6) \\
 t &\sim \text{Gamma}(0.001, 0.001) \\
 \tau &\sim \text{Gamma}(0.001, 0.001)
 \end{aligned}
 \tag{1}$$

where W_i indicates the error term for i observations with a normal distribution (N) and b specifies the average for each category Y for the parameter θ . The hyperparameters μ and t indicate random variances for the parameter b .

The Poisson model follows as:

$$\begin{aligned}
 W_i &\sim \text{Poisson}(\lambda_i) \\
 \log(\lambda_i) &= b[Y_i] \\
 b &\sim N(\mu, t) \\
 \mu &\sim N(0, 0.10E-6) \\
 t &\sim \text{Gamma}(0.001, 0.001)
 \end{aligned} \tag{2}$$

where W_i indicates the error term for i observations with a Poisson distribution and b specifies the average for each category Y for the parameter λ . The hyperparameters μ and t indicate random variances for the parameter b , specified by an uninformative prior distribution. As in the precedent model, uninformative priors were assigned for all the parameters.

2.3.2 Sample size suitability

For measuring the suitability of the sample size, we used the same models of the probability differences described above. However, we ran the models several times, randomly taking away one replicate from the dataset each time.

The Cohen d effect size (1988) was calculated to measure the impact of reducing the sample size in data. It was considered a value of 0.2, 0.5 and 0.8 for the small, medium and large effects, respectively (Cohen 1988).

2.4. Software and Simulations

The models were generated through the online web tool BayesBehav available at <https://euriconoleto.shinyapps.io/BayesBehav>. This webtool makes easier to implement Bayesian longitudinal models through the R statistical package “rjags” (Plummer et al. 2006) and the software JAGS (Just Another Gibbs Sampler) (Plummer 2003).

The parameters were estimated through simulations by using trial and error in the model convergence process. The criterion of Gelman-Rubin (Gelman & Rubin 1992) was used to check the success or failure in convergence. It was also used for establishing a period of “burn-in” constituted by the disposal of some chains to help the model convergence.

3. Results

All species presented variables with very low frequencies that were not suitable to be modeled. Thus, we described only the variables with enough frequencies to generate reliable results. For all the species, only one or two variables provided similar results to the combined variables. Within this context, the feasibility of reducing the sample sizes was made only for the most frequent variables, since using only these variables would invariant the study conclusions, as described below. Moreover, for sake of simplicity, the results of the species *P. scalare* & *O. niloticus*; and *C. paranaenses* & *A. ocellatus*, were presented in pairs due to similar outcomes from them.

3.1. Combining variables

P. scalare and *O. niloticus*

For the *P. scalare* and the *O. niloticus*, concerning the more energetic behaviors, the variable attacks (A) was defined as major variable, while chase (C) and bites (B) as minors. When combining these two minor variables in A category, the resulting distribution was strongly influenced by the B one, for both species (Figures 1A, B, C and 2A, B, C). It has similar patterns, close means values and probabilities, although this last may change in 1% to 3% (*P. scalare*) or 3%

to 7% (*O. niloticus*), depending on the species. For *P. scalare*, when comparing the major variable attacks with the minor variable bites, differences among the second-third and the fourth-fifth days decreased from 77% to 74% and from 94% to 92%, respectively (Figure 1B, C). In addition, differences among the third-fourth day increased from 73% to 76%. Both variables increased from the first to the second day, decreasing until the fifth. The variable chase presented consistency, not changing in time (Figure 3A). For *O. niloticus*, chase and bites presented an increasing pattern until the third day and a decreasing trend until the fifth (Figures 2B and 4A). For this species, when comparing the major variable attacks with the minor variable bites, differences among the first-second, the first-third, the second-third, the third-fourth and the fourth-fifth days decreased from 92% to 87%, 95% to 92%, 72% to 64%, 90% to 84% and 87% to 80%, respectively (Figures 2B, C). The differences among the first and third days were the only ones that affected the statistical significance.

A similar pattern was recorded for the major variable displays (D) in both species, where the minor variables lateral threat (LT) and undulation (U) stands out (Figure 1 and 2). However, LT was the variable which influenced the distributions of all days recording similar patterns, close means values, and probabilities (Figures 1D, E, F and 2D, E, F). For both species, the variable undulation presented consistency, not changing in time, and very low frequencies (Figures 3B and 4B). For *P. scalare*, the only probability that changed was the one between the fourth and fifth day, which is 86% using only lateral threat (LT) and 91% using all variables (Figures 1E, F). For *O. niloticus*, when comparing the major variable displays with the minor variable LT, differences among first-second, second-third and fourth-fifth days increased from 91% to 95%, 92% to 95% and 64% to 72%, respectively (Figure 2E, F). Hence, combining variables generated a lost in the statistical significance in some days (first-second and second-third).

For the major variable attacks of the *A. ocellatus*, two minor variables stand out: the variables chase (C) and bites (B). On the other hand, four minor variables stand out for the *C. paranaense*, such as lateral attacks (LA), chase (C), push (P) and tail beating (TB). However, when these variables were combined in attacks, the resulting distribution was remarkably influenced by the variable lateral attacks for both species. Indeed, LA recorded similar patterns, close mean values and probabilities (Figures 5 and 6). Although, the mean values are lower than attacks (A), and probabilities may change in 2 to 8% (*C. paranaenses*) or in 1 to 18% (*A. ocellatus*), depending on the species. For *C. paranaenses*, when comparing the major variable A with the minor variable LA, differences among the second-third and the third-fourth days increased from 54% to 62% and from 68% to 70%, respectively (Figure 5B, C). For this species, the variables lateral attacks, chase and push presented similar patterns decreasing from the second day. However, the last two variables presented much lower frequencies compared to the first one (Figure 5B and 7A, B). The tail beating (TB) presented a pattern of consistency, not changing in time, and low frequencies (Figure 7C). For *A. ocellatus*, the LA presented a decreasing pattern until the second day and then an increasing trend until the fifth day (Figure 6B). The chase increased from the third until the fifth day (the second day has no frequencies in this variable), with very low frequencies compared to the lateral attacks (Figure 6C). For this species, when comparing the major variable attacks with the minor variable LA, differences among the first-third and the third-fifth days decreased from 97% to 78% and from 100% to 88%, respectively. Therefore, a statistical significance (more than 95% of difference) is revealed when combined variables are used, but not when lateral attacks is implemented individually. For the major displays, both species presented variables with very few frequencies. As a matter of fact, only the variable lateral threat from the *C. paranaenses* could be properly modeled. These variables presented an increasing pattern until the fifth day. As only one variable was suitable to be modeled, probabilities did not change even by adding the very few frequencies from other variables.

3.2. Family distribution

For *P. scalare*, models with a Gaussian distribution provided better results (based on lower DIC values) than using the Poisson one for most variables (Table 2). However, for undulation and chase, Poisson models provided better fits. Similarly, for *O. niloticus* and *C. paranaenses* Gaussian distribution models were better than the Poisson ones in all variables (Table 2). Based on these findings, Gaussian distribution models were more suitable for most species, except for the *A. ocellatus*, for which the Poisson models were better.

3.3. Reducing sample size

P. scalare and *O. niloticus*

For the *P. scalare* and the *O. niloticus*, reducing the sample sizes was done in the two main minor variables, lateral threat (LT) and bites (B), and in the two major variables, displays (D) and attacks (A). For the LT and the D the reduction of the sample size tended to decrease the posterior probability of difference among all the days, for both species. The only exception was found between the third-fourth and four-fifth day for the *P. scalare* and *O. niloticus*, respectively. At the inference among these days, the posterior probability did not present a clear pattern, also showing a small effect size (Table 3). For *P. scalare*, in both variables, the difference within the first three days remained the same until the tenth replicate ($\neq 100\%$) (Figure 8A), presenting a large effect size (Table 3). For *O. niloticus*, in both variables, the difference among the first four days remained the same until the twelfth replicate (Figure 8B). For the LT this difference was of 95% within the first-second and second-third days and 100% within the third and fourth days. For the D this difference was of 91%, 93% and 98% within the first-second, second-third and third-fourth days, respectively. The effect size between these days, for both variables, was considered medium (>0.5) and large (>0.8) (Table 3).

For bites and attacks of the *P. scalare*, the reduction of the sample size tended to decrease the posterior probability of difference among the first and second day, and the fourth and fifth day (Figure 8A). Between these days there was as medium effect size (Table 3). From the second until the fourth day there was no clear pattern, with a small effect size was found for both variables (Figure 8A and Table 3).

For B and A of the *O. niloticus*, the reduction of the sample size did not present a clear pattern within any of the days. It is noteworthy that the sample size reduction increased the probably of difference for both variables, from the fifteenth until the eleventh replicate, while decreased until the fifth (Figure 8B). For the A variable, this increase generated a non-significant result with fifteen replicates ($\neq 90\%$), which become significant with eleven replicates ($\neq 95$). The differences within all days, for both variables, presented a small effect size (<0.5) (Table 3).

C. paranaenses and *A. ocellatus*

For the *C. paranaenses*, reducing the sample sizes was done in the two main minor variables, lateral threat (LT) and lateral attacks (LA), and in the two major variables displays (D) and attacks (A). For the LT and the D, the reduction of the sample size did not present a clear pattern within most of the days (Table 3), also presenting a small effect size. The only exception was found between the first and second day, in which the reduction of the data sample size tended to decrease the posterior probability of difference. For both variables, the difference among the first and second day remained the same until the eleventh replicate ($\neq 100\%$) and decreased until the rest of the sample reduction (Figure 8C). The effect size between this inference was considered large (≥ 0.8) (Table 3). Similar to the LT and the D, the LA and the A did not present a clear pattern within most of the days, with a small effect size. The only exception was in the inference among the first and second day, which invariant in the sample reduction with a large effect size (Table 3).

For the *A. ocellatus*, reducing the sample sizes was done in the main minor variable, LA. For this variable the reduction of the data sample size invariant the probability of difference within

most of the days. The unique exception was found between the fourth and fifth day, in which the posterior probability tended to decrease. This difference remained the same until the ninth replicate ($\neq 100\%$) and decreased until the rest of the sample reduction (Figure 8D). The effect size was not calculated for this species, since modeling was done with Poisson distribution.

4. Discussion

4.1. Combining Variables

When two longitudinal variables are combined, two patterns are mixed at the same time. Usually, patterns of the most quantitative variables prevail, while the other variable cause small deviances on this trend (Noletto-Filho et al. 2017). For most of the species, the most frequent variable from attacks (A) and displays (D), provided individually similar results compared to using combined variables. Although, there were two issues: (i) the mean values were lower; and (ii) the probabilities differences changed from one to eight percentages, in some cases. The first issue may look as an advantage of combining variables, which is to include information from other variables that also existed in the fight. However, even if this fact is considered, only the temporal pattern of the most quantitative variable is represented. Hence, increasing the mean values compared to using a single one. However, these additional frequencies caused small deviances on the pattern of the main variables which leads us to the second issue: deviances generated probabilities gaps, which also caused lost and gain of statistical significance when combining results in some days. Therefore, instead of enriching our analysis with data from other variables, we are actually misleading the information of the main one. Noletto-Filho et al. (2017) have already detected this problem when combining A (more intense aggressive behavior) and D (less intense aggressive behavior) in total attacks, and here we saw that it goes deeper for variables that compose Attacks and Displays by itself. This classification in more (A) and less (D) intense aggressive behaviors was described by Hallen & Wittenberger (1987), where it was noticed the substitution of attacks by displays during cohabitation. Subsequently, it was adopted in other studies (e.g. Gomez-Laplaza & Morgan 1993,

Oliveira & Almada 1998, Werneyer et al. 2002, Galhardo et al. 2008, Desjardins et al. 2012, Balzarini et al. 2014, Barreto et al. 2015). The more intense aggressive behavior (A) are more associated with high energetic costs than the fewer ones (D), regardless the frequencies of aggressive events in each category (Ros et al. 2006). In other words, a low frequent aggressive behavior can have more impact on the metabolism than a more frequent one. Consequently, combining variables can hide the pattern of the variable with more impact on the metabolism, misleading information from the experiments. Within this context, it is still interesting to categorize the variables by its energetic costs, although without combining them.

The only species that presented more remarkable differences among the variable combined and the most quantitative variable was the *A. ocellatus*. For this species, combining variables increased in up to 18% the difference of some days compared to using the more quantitative variable. This species presented the lowest mean and standard deviations in variables among all species in our study, which is probably the reason why it was more susceptible to deviances when combining variables. For example, if 50 lateral attacks are combined with 2 bites, there is 3% increase in the amount of aggressive behaviors and it may not be considered a big change. However, if instead only two lateral attacks are combined with 2 bites, there is a 100% increase in the amount of aggressive behaviors and, this time, it is a big change. Hence, combining variables will generate more or fewer deviances from its minor variables depending on its frequencies.

Combining aggressive variables for data analysis is also done for other animals such as mice, birds, bears, lobsters, and others (e.g. Van Loo et al. 2002, Sima et al. 2015, Frederick et al. 2013, Ayres-Peres 2015, respectively). Therefore, it is possible that the same problem could appear in these studies. The Bayesian method could also be applied to verify the feasibility of combining in those cases, because in these studies it is also counted the frequency of aggressive events standardized with an ethogram. Researchers can easily apply the proposed Bayesian method using the BayesBehav web tool at <https://euriconoleto.shinyapps.io/BayesBehav> (Noletto-Filho et al. unpublished).

4.2. Family distribution

The aggressive behavior data can be viewed as a Poisson process because it is composed of counts and discrete data. However, our results highlighted that Gaussian models were more suitable for most of the species (based on DIC values). According to the central limit theorem, Poisson and Gaussian distributions are numerically close when the variables are highly quantitative. However, for the Poisson distribution the mean is equal to its variance, hence it does not account for uncertainty as accurate as the Gaussian model (Ventura et al. 2015). The *A. ocellatus* was the only species whose main variable (LA) was better modeled using the Poisson distribution. This distribution is more suitable to work with low frequencies data and zero-inflated variables (Varona & Sorensen 2010). Hence, it is better to study a species with a low frequency on its aggressive behavior as the tiger oscar.

4.3. Sample size

The required sample size for the longitudinal aggressive behavior data is influenced by the degree of overlapping from the time intervals, which is related to the effect size (Noleto-Filho et al. 2017). With a large effect size, it is possible to detect an effect in smaller sample numbers, whereas a smaller effect size requires larger sample sizes (Sullivan & Feinn 2012). Within this context, it was already expected that the variables with smaller effect sizes would be more sensitive to sample numbers. For example, in the variable Attacks of the *O. niloticus*, the inference among the first-second (effect size = 0.47) and fourth-fifth (effect size = 0.38) days are statistically significant with twelve replicates ($\neq 96\%$), but it is not with fifteen ($\neq 92$). Thus, a small sample number would have provided a false negative result. In general, effect sizes bigger than 0.7 (medium to large effects) invariant results until the 12^o replicate, for the minor variables of all species. However, all species presented at least one variable with small and large effect sizes within the inference of its time intervals. Given that it is unfeasible to disjoint the replicate numbers among time intervals in a longitudinal design, replicates should be based on the inference with the smaller effect sizes. The variable Bites of the *O. niloticus* holds an example of an appropriate sample size for a small effect

size. For this variable, the inference among the first-second days presented a small effect size (0.38), but it only changed 2 percentages from fifteen ($\neq 91\%$) until twelve ($\neq 93\%$) replicates. Hence, such deviance has not changed any conclusion and is an appropriate sample size. The BayesBehav web tool (<https://euriconoleto.shinyapps.io/BayesBehav> Noletto-Filho et al. unpublished) could be used for this purpose, as it was done in the present research (see sections 2.3.2 and 2.4).

In addition, the most frequent variable from attacks and displays, provided individually similar results compared to using variables combined. Hence, it was also already expected that the main minor variables with its respective major variables would respond similarly when reducing its sample sizes. Indeed, the combining variables had no impact in the feasibility of reducing the sample size compared to using the most quantitative minor variable.

5. Conclusions

The results highlight that, when combining behavioral variables, the resulting distribution is remarkably influenced by only one variable for each category. Consequently, aggregating values mislead results in some cases, changing the probabilities from the main variables. In addition, species with low frequency in aggressive behavior are more sensitive to this bias. The variable lateral threat is the main low intensity variable for all species, while bites is the main high intensity variable for the *P. scalare* and the *O. niloticus*. In addition, the variable lateral attack is the one belonging to the high intensity category with more relevance for the *C. paranaenses* and *A. ocellatus*. Combining variables has no impact in the feasibility of reducing the sample size compared to using the most quantitative minor variable. Effect sizes bigger than 0.7 (medium to large effects) invariant results until the 12^o replicate, for the minor variables of all species. Concerning family distribution, the Gaussian model is more suitable for most of the species. The unique exception is the *A. ocellatus*, which is better modeled by Poisson distribution.

6. References

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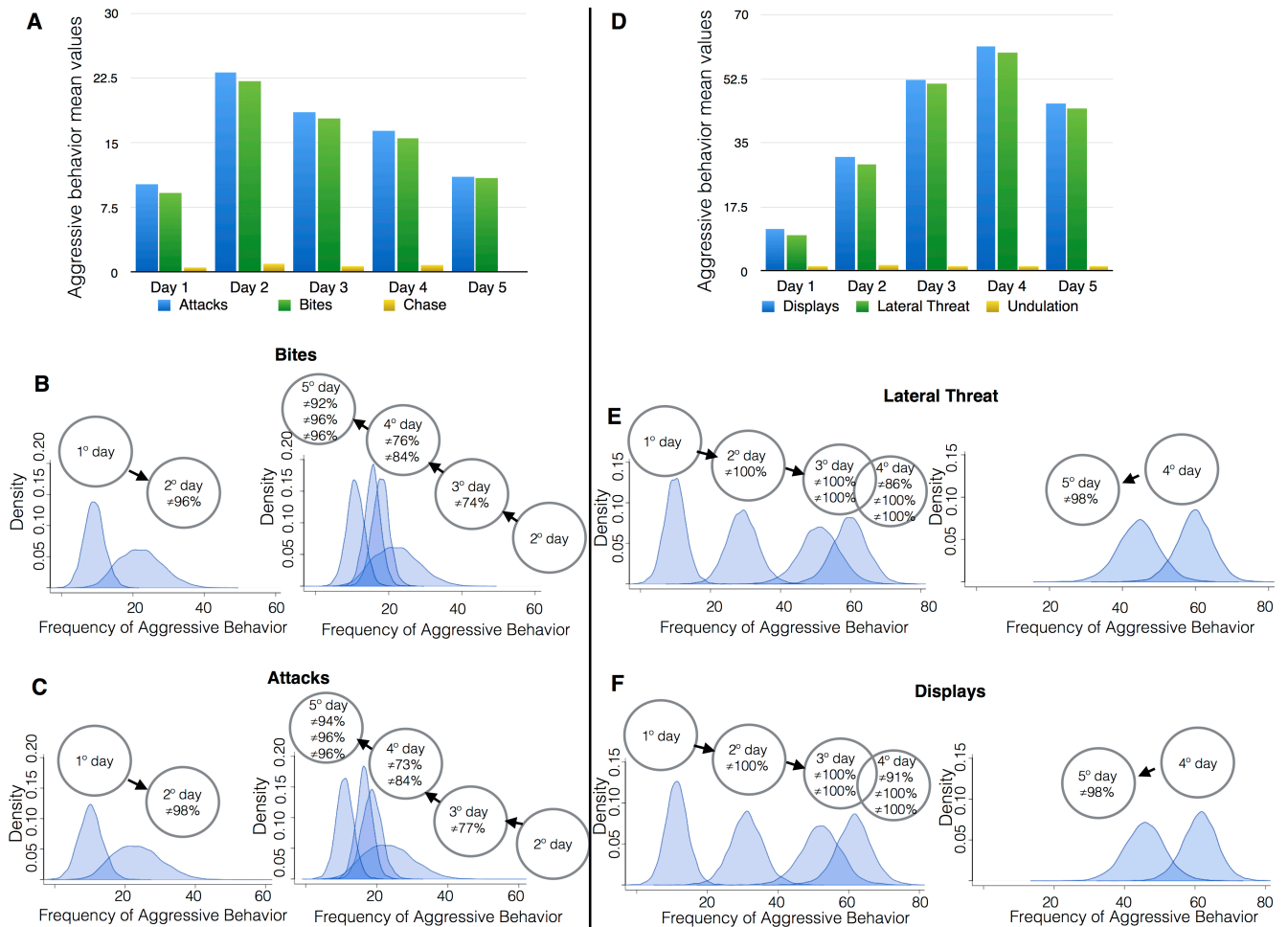


Figure 1: *Pterophyllum scalare* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The mean values of the most frequent high intense aggressive variables, individually (Bites and Chase) and the mean value of them combined (Attacks). B and C - The daily aggressive behaviors density distributions of the Bites (B) and Attacks (C). D - The mean values of the most frequent low intense aggressive variables, individually (Lateral threat and Undulation) and the mean value of them combined (Displays). E and F - The daily aggressive behaviors density distributions of the Lateral threat (E) and Displays (F). The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

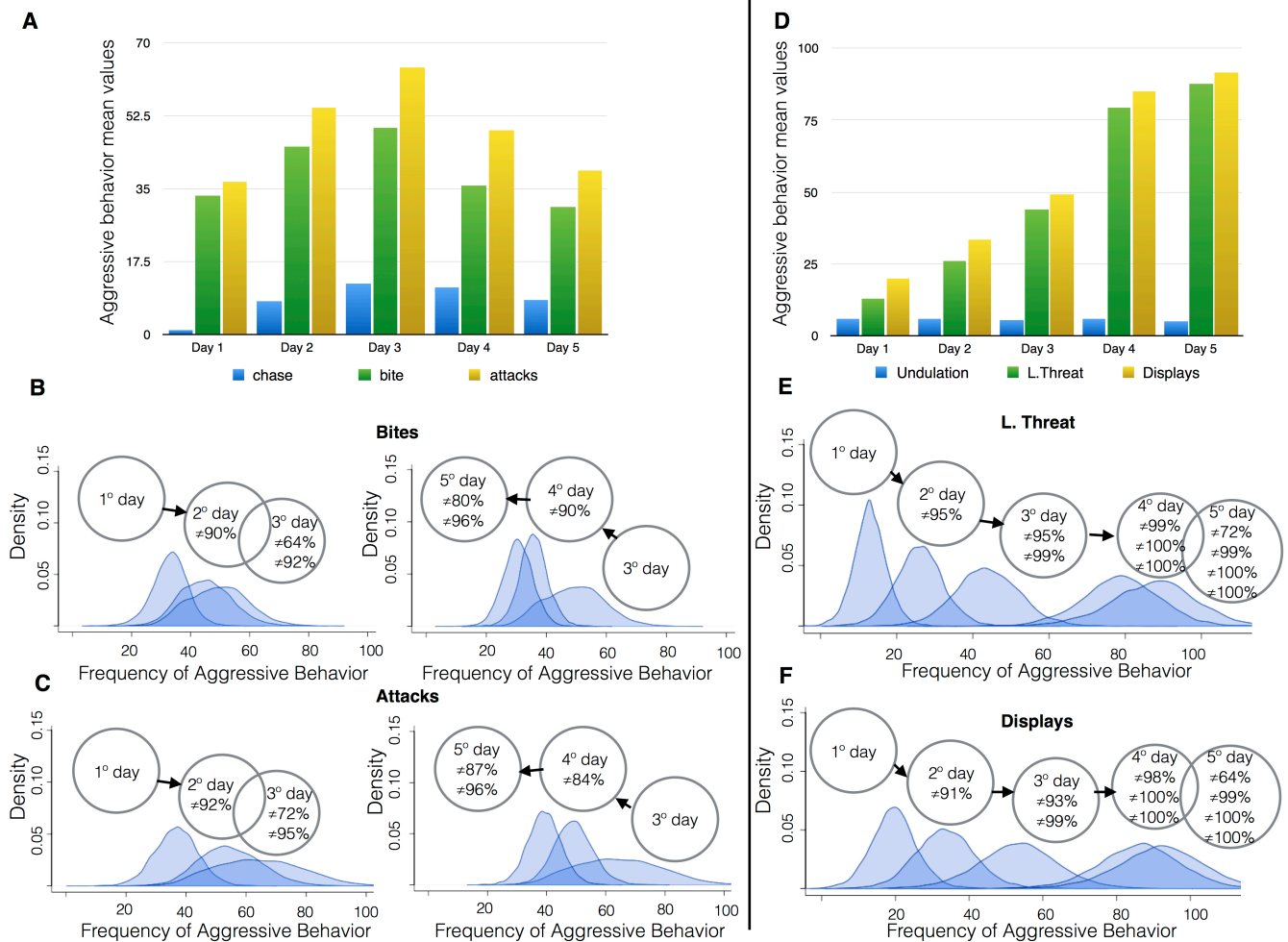


Figure 2: *Oreochromis niloticus* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The mean values of the most frequent high intense aggressive variables, individually (Bites and Chase) and the mean value of them combined (Attacks). B and C - The daily aggressive behaviors density distributions of the Bites (B) and Attacks (C). D - The mean values of the most frequent low intense aggressive variables, individually (Lateral threat and Undulation) and the mean value of them combined (Displays). E and F - The daily aggressive behaviors density distributions of the Lateral threat (E) and Attacks (F). The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

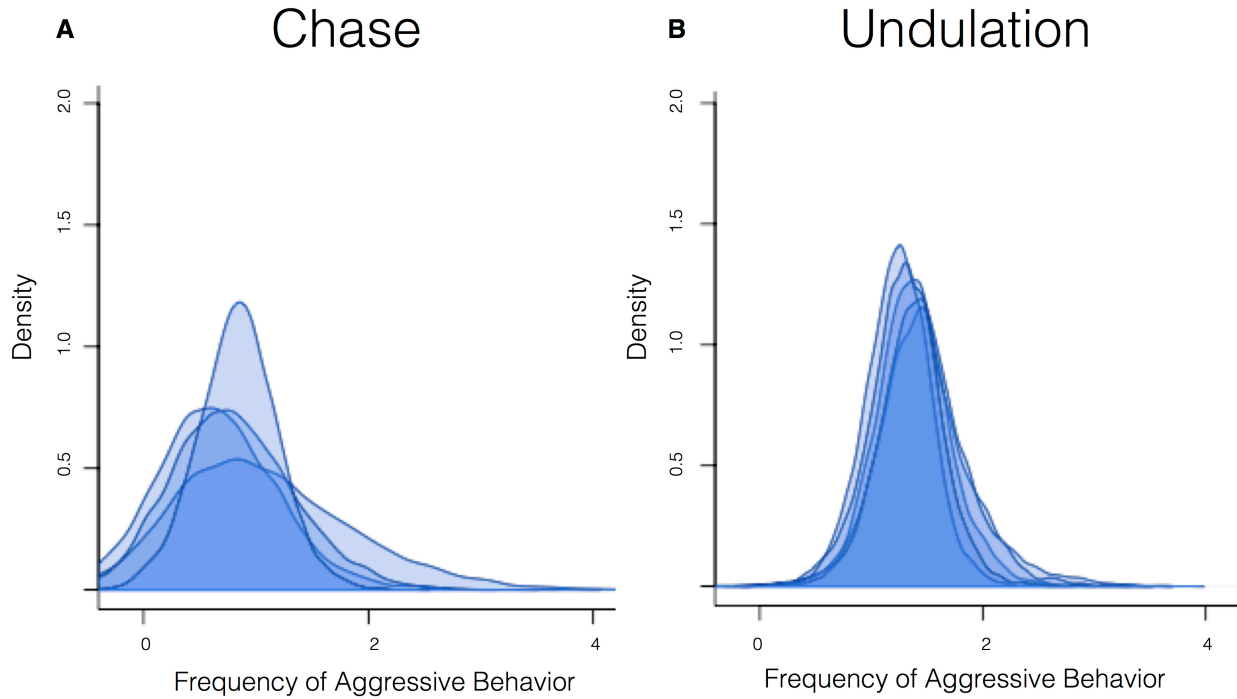


Figure 3: *Pterophyllum scalare* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The daily aggressive behaviors density distributions of the Chase. B - The daily aggressive behaviors density distributions of the Undulation. The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

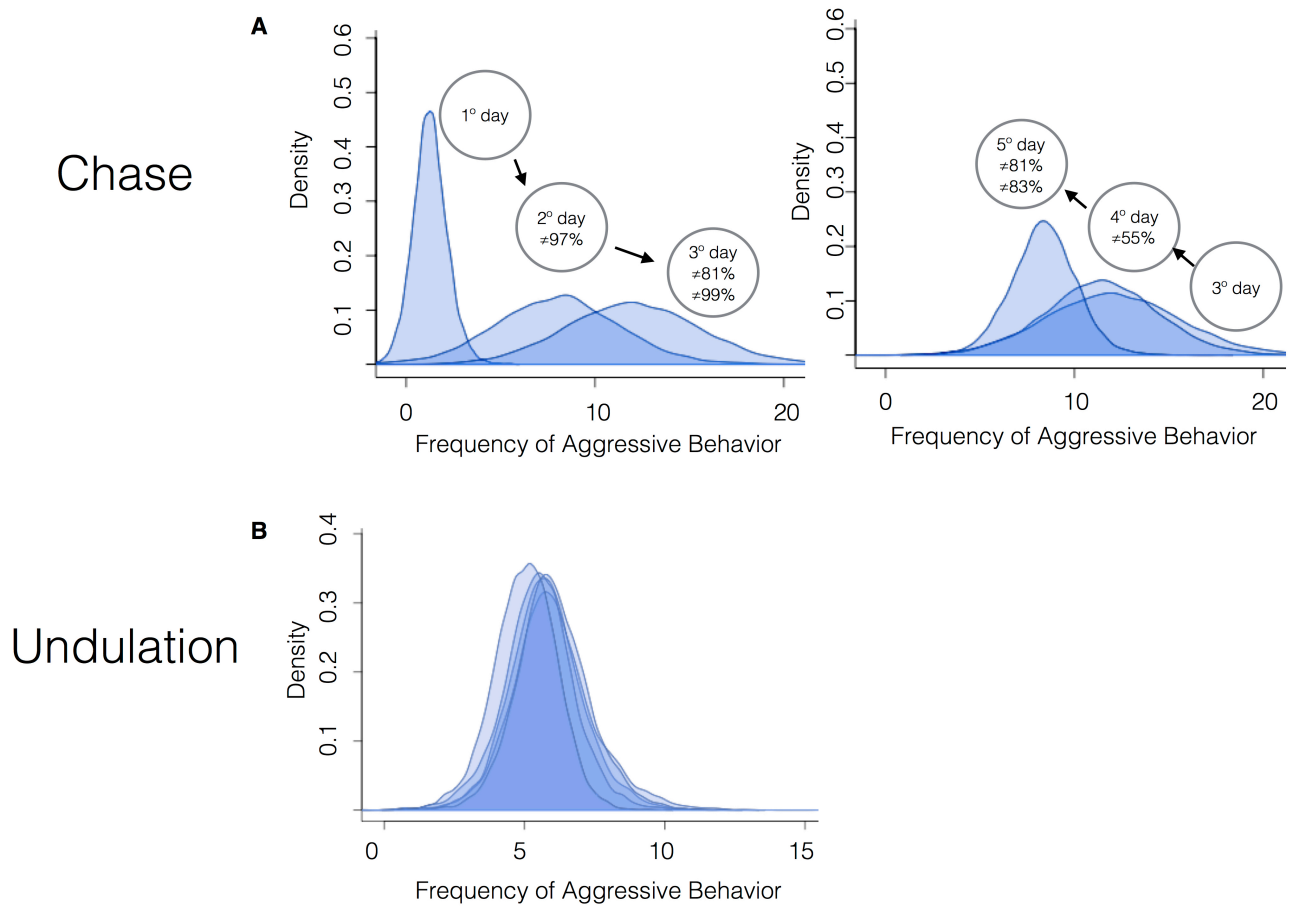


Figure 4: *Oreochromis niloticus* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The daily aggressive behaviors density distributions of the Chase. B - The daily aggressive behaviors density distributions of the Undulation. The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

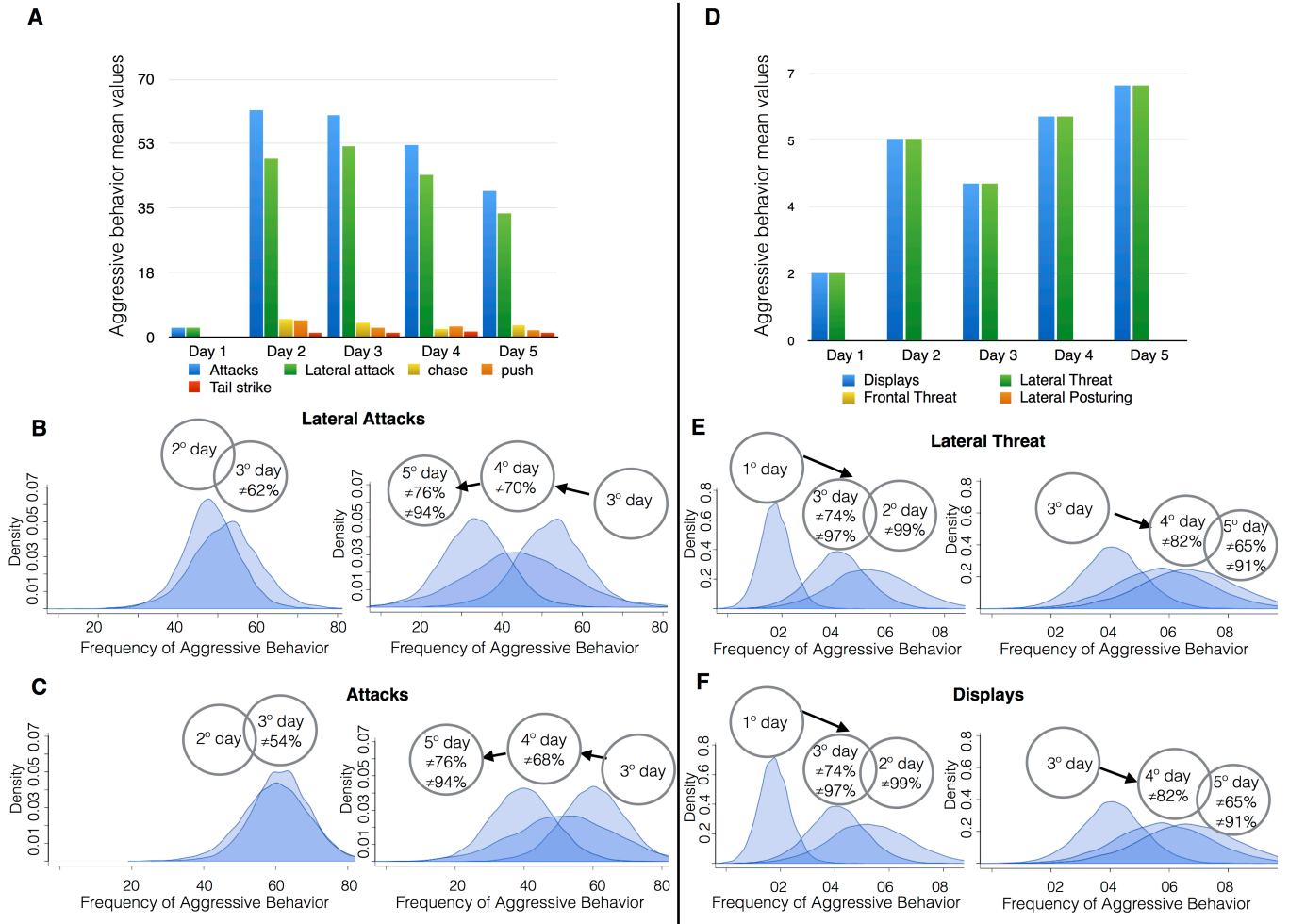


Figure 5: *Cichlasoma paranaenses* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The mean values of the most frequent high intense aggressive variables, individually (Lateral attacks, Chase, Push and Tail beating) and the mean value of them combined (Attacks). B and C - The daily aggressive behaviors density distributions of the Lateral attacks (B) and Attacks (C). D - The mean values of the most frequent low intense aggressive variables, individually (Frontal threat, Lateral threat and Lateral posturing) and the mean value of them combined (Displays). E and F - The daily aggressive behaviors density distributions of the Lateral threat (E) and Attacks (F). The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

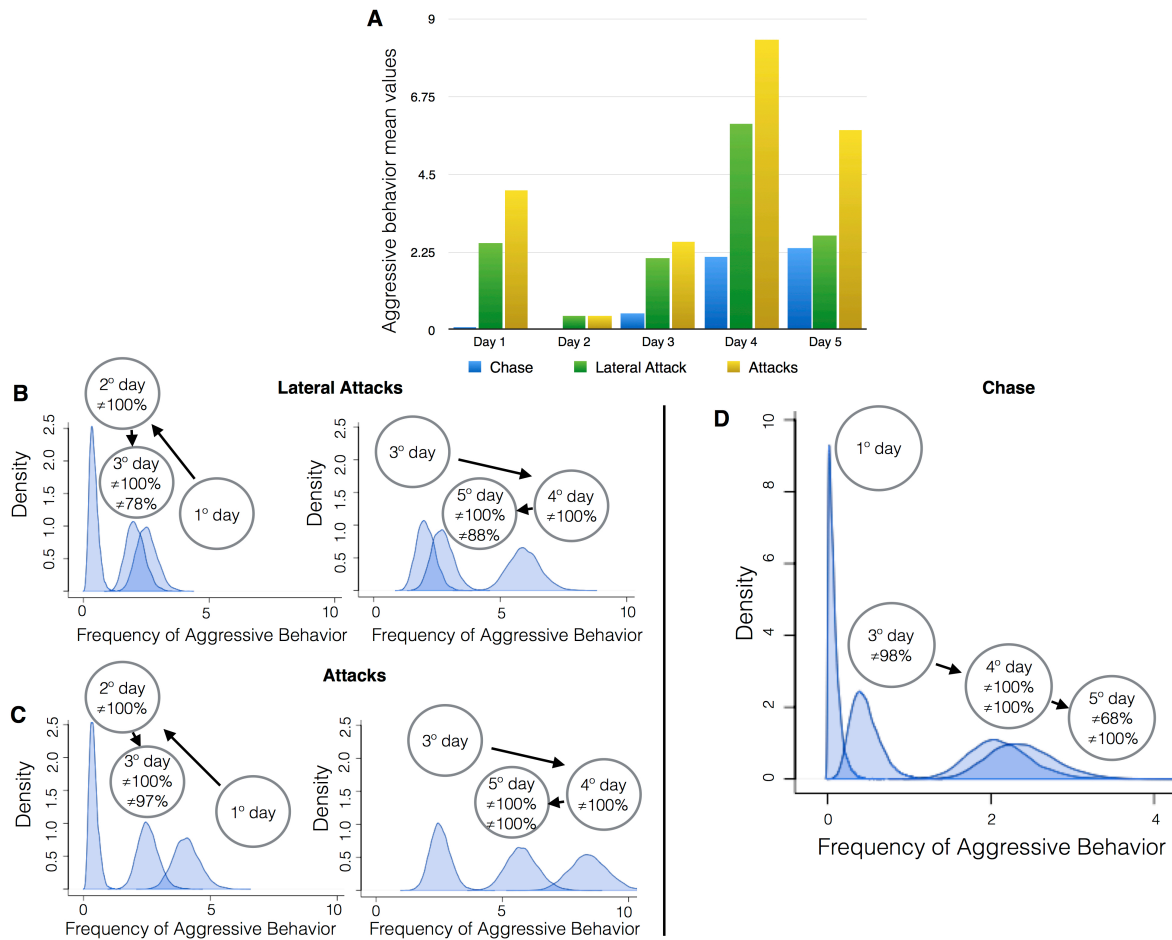


Figure 6: *Astronotus ocellatus* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The mean values of the most frequent high intense aggressive variables, individually (Lateral attacks and Chase) and the mean value of them combined (Attacks). B and C - The daily aggressive behaviors density distributions of the L. attacks (B) and Attacks (C). D - The daily aggressive behaviors density distributions of the Chase. The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

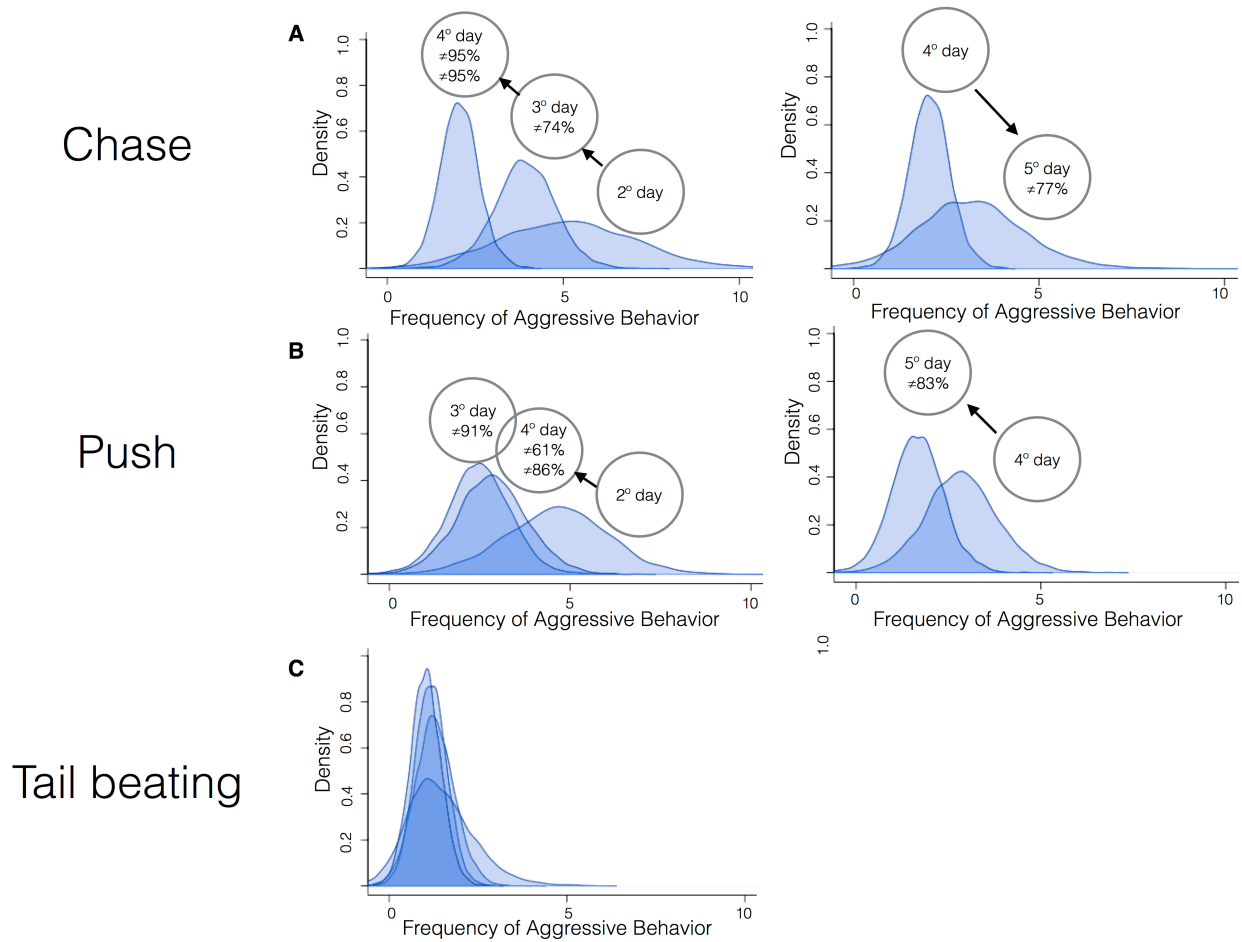


Figure 7: *Cichlasoma paranaenses* aggressive interaction in 5 days period. It was observed in 15 minutes at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on. A - The daily aggressive behaviors density distributions of the Chase. B - The daily aggressive behaviors density distributions of the Push. C - The daily aggressive behaviors density distributions of the Tail beating. The density (y-axis) represents the probability density function per unit of aggressive behavior (x-axis).

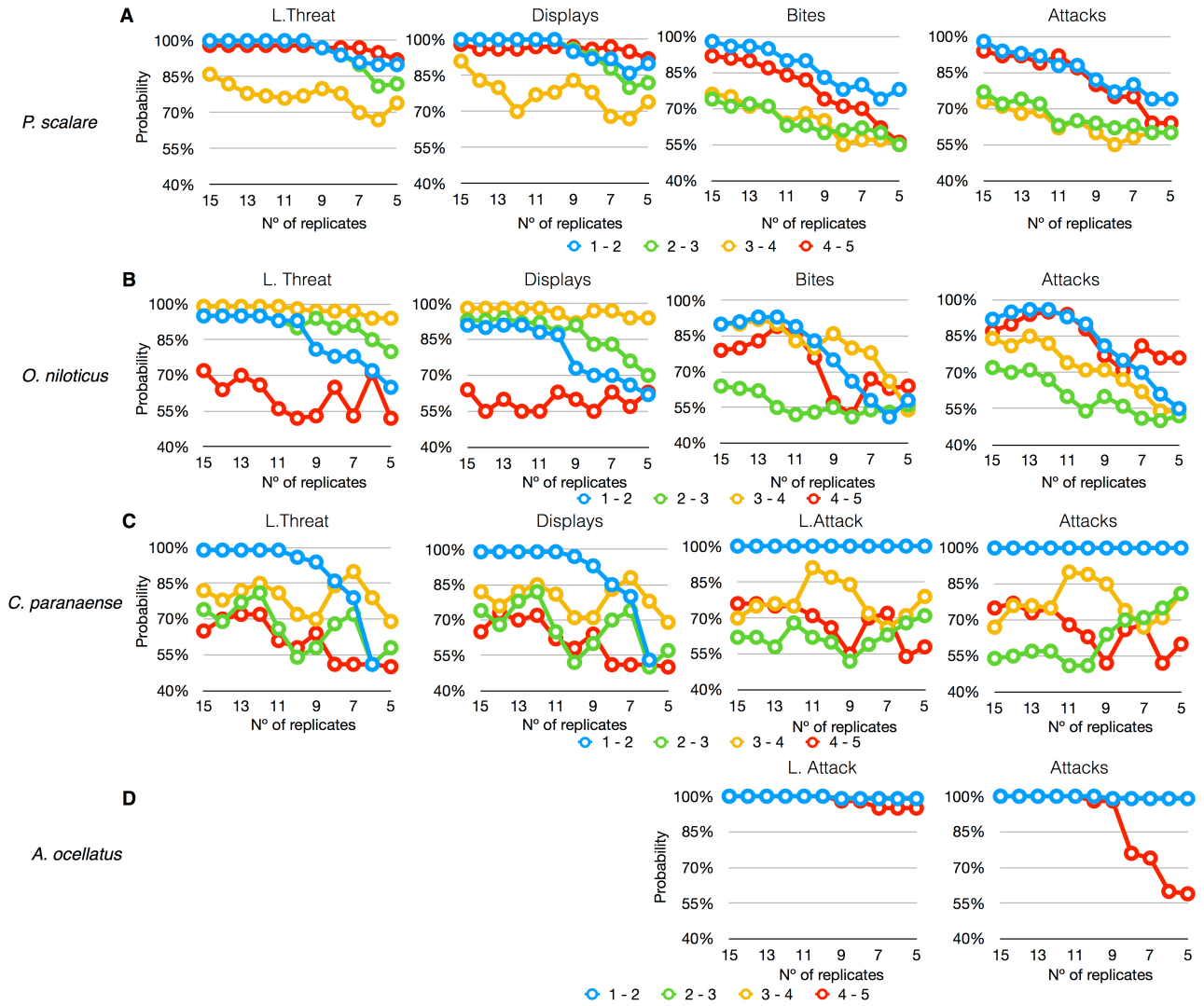


Figure 8: The distribution of difference between days with different sample sizes of four cichlids species. The species illustrated are the *P. scalare* (A), the *O. niloticus* (B), the *C. paranaenses* (C) and the *A. ocellatus*. The blue line is between the first and second day, the green line is between the the second and third days, the yellow is between the third and fourth days and the orange is between the fourth and fifth days.

Table 1: Aggressive behavior in four cichlid species (adapted from Carvalho et al. 2012, Gonçalves-de-Freitas & Mariguela 2006, Brandão et al. 2015). For ✓ - aggressive event is present. For 0 - aggressive event is absent.

Agonistic event	Species			
	<i>P. scalare</i>	<i>C. paranaense</i>	<i>A. ocellatus</i>	<i>O. niloticus</i>
Attacks				
Chase: one fish follows the opponent that swims in opposite direction.	✓	✓	✓	✓
Tail beating: The tail is quickly and sharply struck against the stimulus. Tail must actually contact divider to be scored.	0	✓	0	0
Lateral attack: the fish remain alongside each other facing the same or opposite direction and beat their tails sideways.	0	✓	✓	✓
Bites: the aggressor swims toward the opponent and bites its body.	✓	✓	✓	✓
Push: A fish with its open mouth nips the lateral flank of its opponent, sometimes displacing the opponent	0	✓	0	0
Displays				
Circling: two fish with erected dorsal fin swim following each other, describing a circle, like a very slow chasing.	0	0	0	✓
Frontal display: both fish approach frontally each other with head-up posture (around 45°), but without physical contact.	✓	0	✓	0
Lateral threat: one fish with their fins spread and mouth opened approaches laterally to the opponent, which keeps away.	✓	✓	✓	✓
Undulation: only one fish beats its tail side- ways (undulating the body), without spreading its fins.	✓	0	0	✓

Table 2: Deviance Information Criterion values from two family distributions (Gaussian and Poisson), of the variables of four cichlids species.

		Gaussian Deviance Information Criterion (DIC)	Poisson Deviance Information Criterion (DIC)
<i>P. scalare</i>	Bites	587	908
	Chase	212	204
	Attacks	656	987
	L.Threat	652	983
	Undulation	320	291
	Displays	600	978
<i>C. paranaense</i>	L. Attack	682	1888
	Chase	252	455
	Push	335	351
	Tail strike	285	290
	Attacks	698	2018
	L.Threat	456	563
	Displays	456	563
<i>O. niloticus</i>	Bites	709	1655
	Chase	548	915
	Attacks	745	2056
	L. Threat	723	1841
	Undulation	520	729
	Displays	764	2182
<i>A. ocellatus</i>	L. Attack	154	123
	Chase	NaN	396
	Attacks	617	650

Table 3: Effect size values of the inference of the main time intervals from three cichlids species. Effect sizes were calculated only for the species modeled using the Gaussian family distribution.

		Effect size between days			
		1-2	2-3	3-4	4-5
<i>P. scalare</i>	Bites	0.67	0.24	0.25	0.51
	Attacks	0.64	0.24	0.20	0.60
	L.Threat	1.30	1.10	0.40	0.75
	Displays	1.26	1.94	0.45	0.75
<i>C. paranaense</i>	L. Attack	2.50	0.11	0.16	0.23
	Attacks	2.73	0.04	0.15	0.24
	L.Threat	0.74	0.21	0.31	0.12
	Displays	0.74	0.21	0.31	0.12
<i>O. niloticus</i>	Bites	0.38	0.12	0.48	0.27
	Attacks	0.47	0.20	0.36	0.38
	L. Threat	0.65	0.62	0.98	0.21
	Displays	0.50	0.52	0.76	0.12
<i>A. ocellatus</i>	L. Attack	NaN	NaN	NaN	NaN
	Chase	NaN	NaN	NaN	NaN
	Attacks	NaN	NaN	NaN	NaN

CAPÍTULO IV

BAYESBEHAV: A BAYESIAN WEB TOOL FOR ANALYZING LONGITUDINAL DATA IN AGONISTIC BEHAVIOR STUDIES

BayesBehav: A bayesian web tool for analyzing longitudinal data in agonistic behavior studies.

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Summary

1. Understanding aggressive behaviors can benefit fish resources management, but still requires overcoming the gap between mathematical models and experimental data. The Bayesian approach offers a coherent framework to help in this task as can account for multiple sources of uncertainty, analyzing longitudinal data and small size samples.
2. Here, we present the BayesBehav, a web application to produce Bayesian analysis for longitudinal studies. This tool has been developed to allow users not familiar with programming languages such as R and Python, to have access to the advantages of Bayesian analysis in longitudinal aggressive behavior studies.
3. This is the first tool designed to apply Bayesian analysis in the context of aggressive behavior, although it can be extended to other research fields that also uses longitudinal designs.
4. We discuss the advantages and limitations of using BayesBehav from the users and developers perspective. The tool can be accessed online at <http://euriconoleto.shinyapps.io/BayesBehav>. The complete code can be freely downloaded from the BayesBehav web site (<https://github.com/euriconoleto/BayesBehav>), under a GPLv3 license.

Key-words: Aggressive behavior, Bayesian analyses, Longitudinal studies.

Introduction

With the continued growth of the aquaculture and fishery industries, the scientific research into the welfare of cultured and wild fishes is vital. In particular, several fish species interact aggressively to set up a dominance hierarchy and defending territory. Understanding aggressive behaviors can benefit fish's management, but still requires overcoming the gap between mathematical models and experimental data. Indeed, different issues arise when aggressive behavior data are analyzed such as: *(i)* replicates are difficult to produce; *(ii)* the behaviors are usually individual-dependent; *(iii)* the experimental designs are longitudinal (*i.e.* concerns the same individual over time).

The use of classical methods to account to these issues could be problematic as imply the respect of several parametrical assumptions as normality, homoskedasticity, and sphericity. In addition, when multiple comparisons are performed using post-hoc tests, the probability that a researcher wrongly conclude that there is at least one statistically significant effect across a set of tests, increases additively with each test (Gelman 2012).

Within this context, Bayesian methods are grown in popularity highlighting their usefulness to solve the mentioned problems in many biological fields including behavioral studies (e.g. McNamara & Houston 1980, Alterovitz et al. 2007, McCarthy 2007, Kinas & Andrade 2007, McNamara & Olsson 2006).

In particular, Noleto-Filho et al. (2017) showed that Bayesian HLMs can offer several advantages with respect to the classical methods as: *(i)* avoid biases, helping with decisions in whether or not combining variables and sample sizes; *(ii)* provide better interpretations to understand changes in time, even when they are subtle to detect; *(iii)* implement robust models that can overcome the heteroskedastic issue.

Among the softwares that are capable to perform Bayesian analysis, most of them require high programming skills (e.g R software - R Development Core Team 2016; Python and; Stan - Carpenter et al. 2017), although some exceptions exist (e.g. Jasp - Marsman & Wagenmakers 2016).

Still, the softwares that need programming have the advantage of being more plastic with specific statistical packages that cope the needs required by different scientific fields. Here we present BayesBehav, the first statistical web tool designed to apply Bayesian HLM for longitudinal studies concerning aggressive behaviors data. In particular, we used the shiny R package (<http://www.rstudio.com/shiny/>), which make possible to create statistical web tools using R packages. We discuss that the BayesBehav application could provides a suitable tool to spread Bayesian analysis in many biological disciplines that also uses longitudinal designs, allowing all the users to benefit from their innumerable advantages.

Materials and Methods

The BayesBehav has been written in R and BUGs languages. The “rjags” (Plummer et al. 2006), “shiny” (Chang et al. 2017) and “shinydashboard” (Chang 2017) packages are needed as an additional packages. The tool can be accessed online at <http://euriconoleto.shinyapps.io/BayesBehav>. The complete code can be freely downloaded from the BayesBehav web site (<https://github.com/euriconoleto/BayesBehav>), under a GPLv3 license. Users can run the code locally at the command line after installing the two required packages cited above.

Model Convergence

The program uses the Gelman-Rubin criteria (Gelman & Rubin 1992) and the trace plot to check if the model have converged. Users can easily adjust the thin number, adaptations and chains of the models using slide bars (*Figure 1*). Using the BayesBehav will be easier to check the model convergence with respect to R in which the code need to be run multiple times changing the different parameters until achieve the convergence. Indeed, using BayesBehav when a parameter will be changed in the slide bars, the new results will be updated automatically.

Family distributions, variables and intervals

BayesBehav provides two data distributions options, such as normal and poisson. These two distributions were selected as are the most commonly used in the analysis of aggressive behavior data (e.g. Maan et al. 2001, Ros et al. 2006, Sadoul et al. 2016, Slavic et al. 2016).

Three different types of variables and six different time intervals can be implemented when an analysis is performed in BayesBehav. Similarly to the selection of the distributions options, these criteria were fixed as are the most common in most experimental aggressive behavior designs (e.g. Gómez-Laplaza & Morgan 1993, Gonçalves-de-Freitas et al. 2008, Maan et al. 2001, Ros et al. 2006).

Multiple Comparisons

Multiple comparisons are usually performed in aggressive behavior studies for accessing differences within groups (e.g. Terleph 2004, Carvalho et al. 2012). The Bayesian method is appropriate for dealing with multiple comparison, because instead of making confidence intervals wider as in classical estimates corrections, they move point estimates and their corresponding intervals closer to each other (Gelman 2012).

In BayesBahev it is possible to access to the different probabilities within the time periods of the same variable or among different variables. It uses a boolean variable that counts the number of simulations in which the difference among and within days are ≥ 0 or < 0 , representing a Bayesian analogue to the classical p-value approach (Gelman et al. 1996, Kéry & Royle 2016). *Figure 2* shows the inboxes of the BayesBahev application, where: *the light blue infobox* represents the difference from the first and second time interval of the first variable; *the red infobox* represents the difference from the first and second time interval of the second variable; *the green infobox* represents the difference from the first and second time interval of the third variable; *the yellow infobox* represents the difference from the first and second time interval between the first and second variable respectively; *the purple infobox* represents the difference from the first and third time interval between the first and third variable respectively; finally, *the blue infobox* represents the difference from the second and third time interval between the first and third variable respectively.

Graphics

Bar-plots and line graphs are common choices for representing the aggressive behavior data (e.g. Gonçalves-de-Freitas et al. 2008, Maan et al. 2001, Ros et al. 2006). Usually, results are illustrated side-by-side in these graphs. Indeed, this type of organization is deceiving for the human eye when comparing multiple results (Goldberg & Helfman 2010). BayesBehav produces kernel density estimation graphics using one or multiples variables (*Figure 3*). Kernel density estimation is a non-parametric way to measure the probability density function of a random variable. It is similar to histograms, but with smoothing and continuity properties. BayesBehav allows overlapping the distributions improving the visual comparisons. The degree of overlapping indicates if the distribution is more or less likely to differ statistically. In addition, this option allows visualizing how a variable change in time by plotting the distributions of sequential time intervals. This possibility to visualize the time perception is essential as changes can happen in a subtle way, not being noticed by inferences that use only p-values, or just by looking at the experimental data (Noleto-Filho et al. 2017). In *Figure 4*, we illustrate, as an example, the subtle daily changes for the variable attacks of the *Pterophyllum* scalare. Within the graphic we are able to realize that the overlapping degree subtly decreases from the second till the fifth day. Hence, it is a great tool to analyze in detail all the changes happening in the data distributions in time. Finally, color and the line type of the distributions can be also selected in the BayesBehav application.

Known limitations

BayesBehav was designed to work only with longitudinal models, therefore data collected with an independent design could probably provide biased results. BayesBehav is also limited to two data distributions (Poisson and Normal), which excludes some behavioral researches that uses other type of distributions such as binomial data. Additionally, the multi comparison approach only

compares time periods one by one, which can be odd depending on the number of intervals available.

Conclusion

Understanding the longitudinal aggressive behaviors requires overcoming the gap between mathematical models and experimental data. We believe that Bayesian inference provides a powerful tool for fitting longitudinal models in the fish aggressive behaviors research. The BayesBehav web application can provide a suitable tool to this end in a programming language that is widespread in many biological disciplines. We hope that our tool will lower the hurdle to the uptake of this inference approach for empirical biologists. We encourage users to report bugs and provide other feedback to euriconoleto@hotmail.com.

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Authors' contributions

E.M.N.F. and E.G.D.F. conceived the methodology; E.M.N.F. wrote web R application; E.M.N.F., and M.G.P. wrote the package documentation. All authors tested the software, contributed critically to the draft and gave final approval for publication.

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Figures

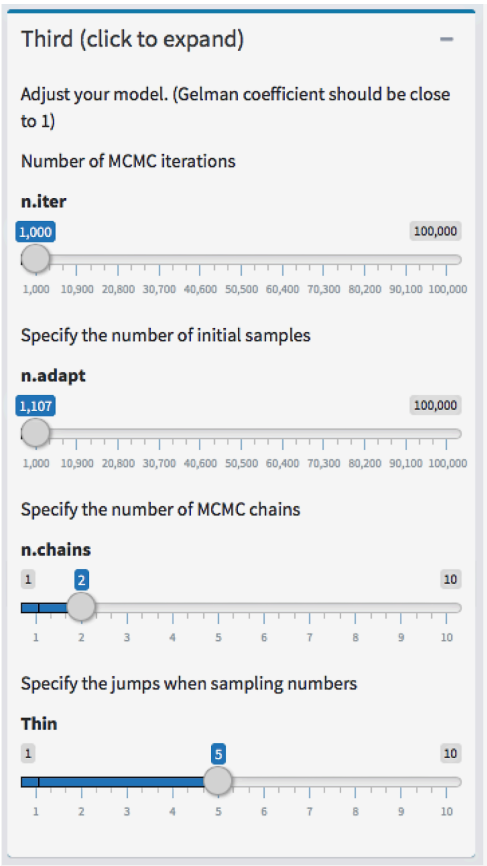


Figure 1: The program Third box from the convergence section allows the user to easy adjust the model through slide bars.

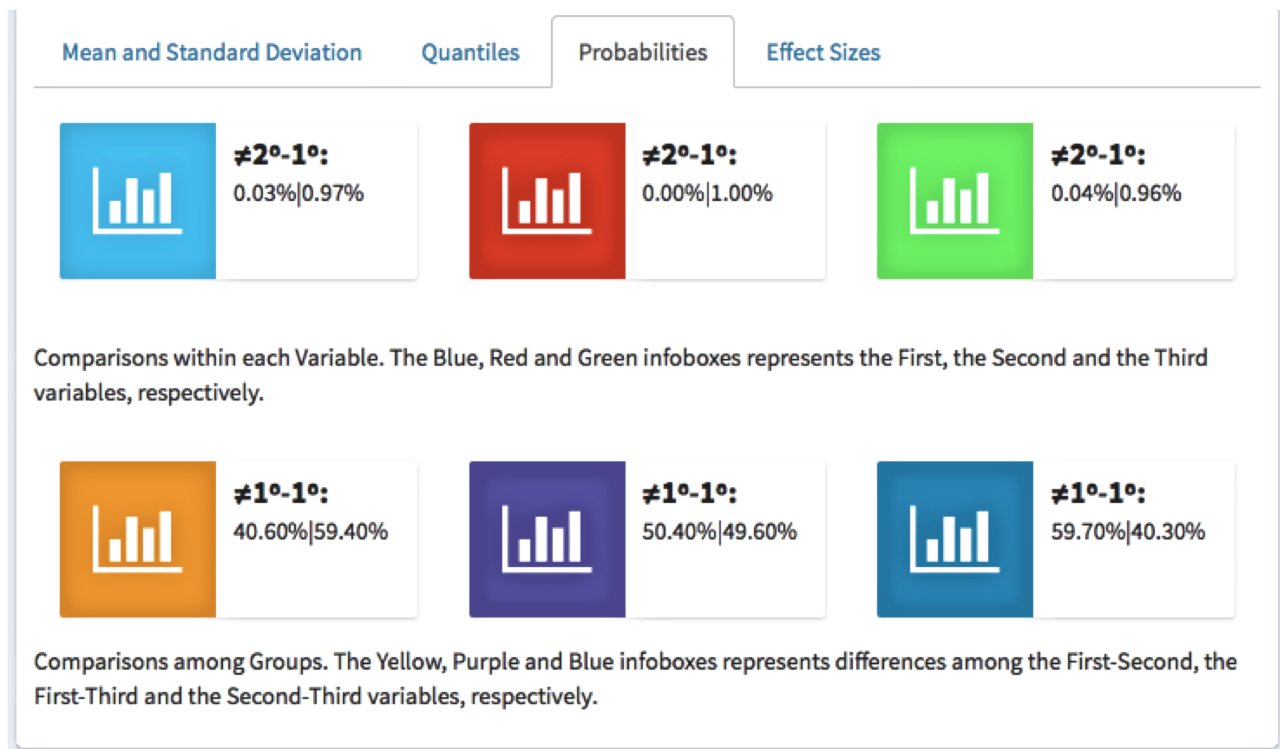


Figure 2: This figure show the results section from of the BayesBehav with *infoboxes* displaying the difference of time intervals among and within variables. The *lightblue infobox* is showing 97% in difference between time intervals within the first variable. The *red infobox* is showing 100% in difference between time intervals within the second variable. The *green infobox* is showing 96% in difference between time intervals within the second variable. The *yellow infobox* is showing 59% in difference between time intervals between the first and second variables. The *purple infobox* is showing 50% in difference between time intervals between the first and third variables. Finally, The *blue infobox* is displaying 60% in difference between time intervals between the second and third variables.

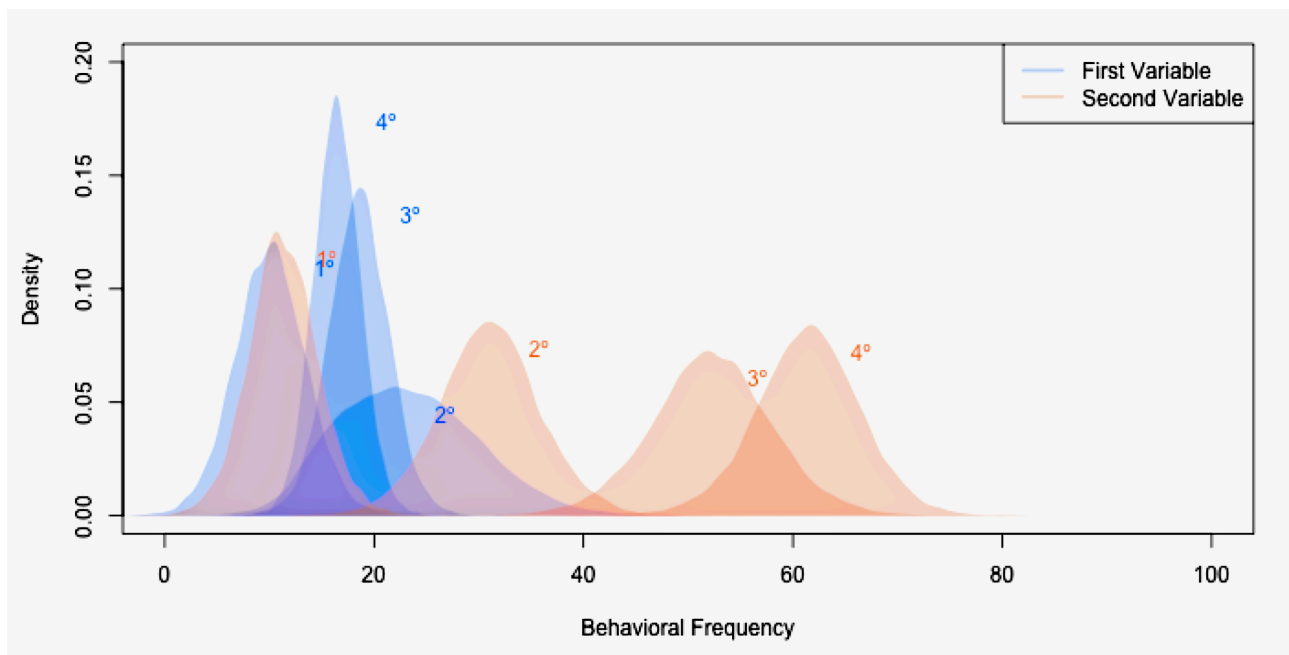


Figure 3: Kernel graph generated by the BayesBehav. It allows plotting multiple distributions of different variables. Users can change color and lines.

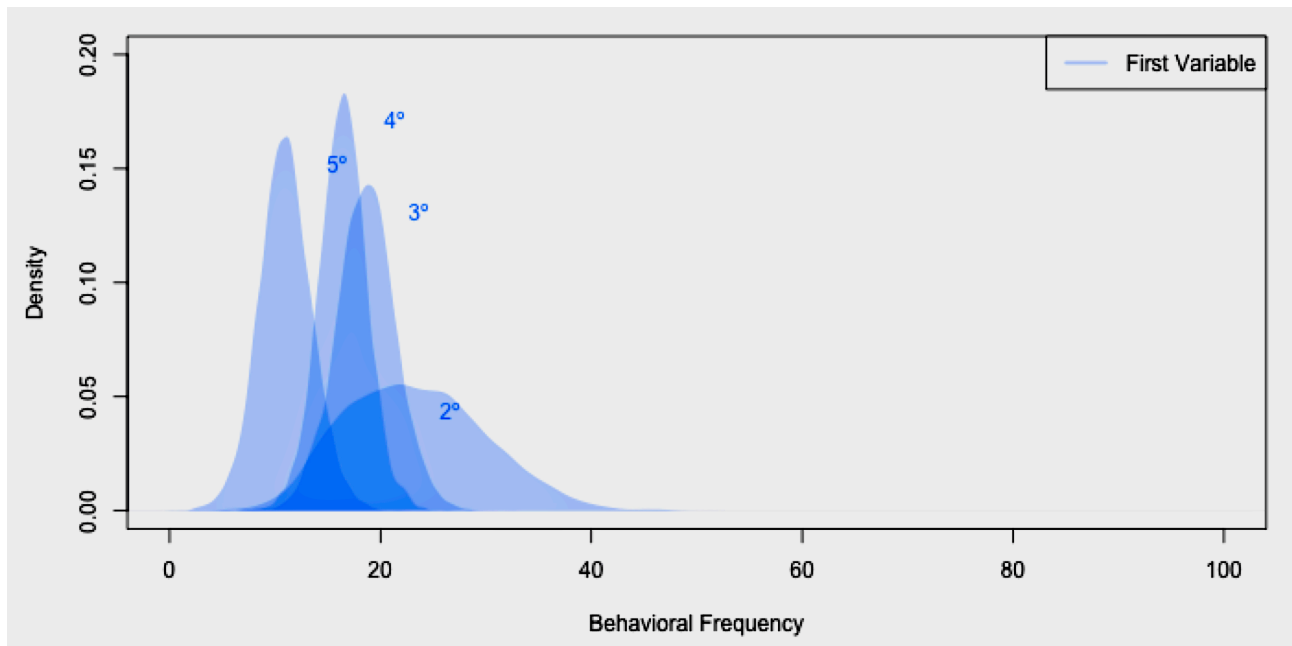


Figure 4: Kernel graph generated by the BayesBehav. It allows to realize that the overlapping degree subtly decreases from the second till the fifth day.

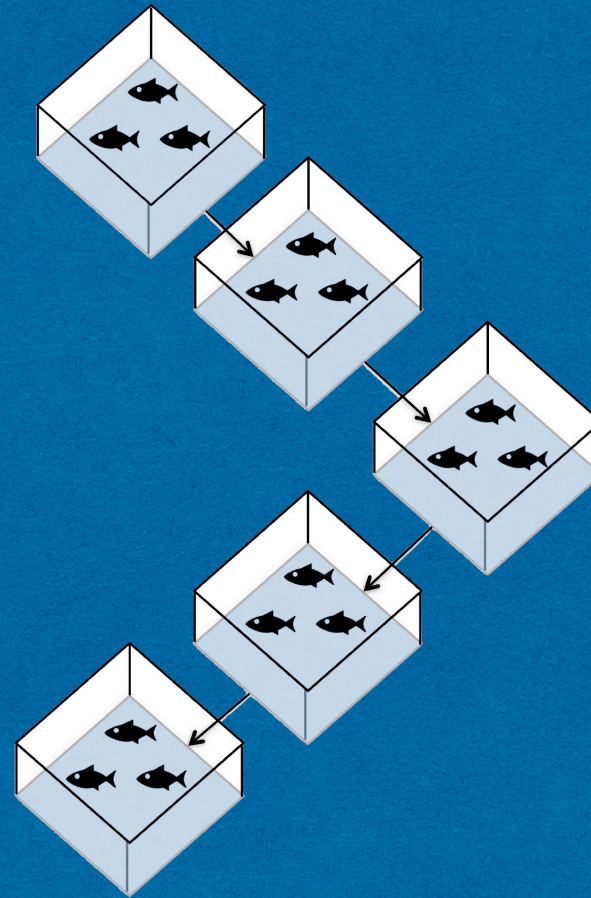
CAPÍTULO V

CONCLUSÕES GERAIS

- Os resultados destacam que ao combinar variáveis comportamentais, a distribuição resultante é notavelmente influenciada por apenas uma variável para cada categoria. Consequentemente, combinar variáveis pode gerar resultados enviesados em alguns casos, alterando as probabilidades das principais variáveis. Além disso, as espécies com baixa frequência em comportamento agressivo são mais sensíveis a este viés. Este pode ocorrer quando agrupar parcial ou completamente as variáveis.
- As mudanças diárias na agressividade podem ocorrer sutilmente não sendo detectadas quando os p-valores são implementados. Pelo contrário, os métodos Bayesianos fornecem uma descrição clara das mudanças mesmo quando os padrões são sutis.
- Quanto à família de distribuição de probabilidade, o modelo Gaussiano foi mais adequado para a maioria das espécies. A única exceção foi o *A. ocellatus*, que é melhor modelado com a distribuição de Poisson.
- Além disso, os resultados mostraram que o número de repetições (15 ou 12) não varia as conclusões do estudo, bem como o uso de um pequeno tamanho de amostra poderia ser mais evidente dentro dos dias sobrepostos para todas as espécies no estudo.
- Portanto, podemos concluir que a abordagem Bayesiana poderia ser um método estatístico mais rico e adequado para estudos longitudinais com comportamento agressivo dos peixes.

MATERIAL SUPPLEMENTAR

BayesBehav: User's Guidelines



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Preface

BayesBehav is a webtool for applying Bayesian Analysis in Longitudinal Studies. It was built to empower users with an easy tool for applying bayesian analysis in behavioral science and related fields. We believe that this inference analysis provides a powerful tool for fitting longitudinal models in behavioral research. The BayesBehav can provide a suitable tool to this end in a programming language that is widespread in many biological disciplines. We hope that our tool will lower the hurdle to the uptake of this inference approach for empirical biologists. BayesBehav can be accessed online at <http://euriconoleto.shinyapps.io/BayesBehav>. The complete code can be freely downloaded from the BayesBehav web site (<https://github.com/euriconoleto/BayesBehav>), under a GPLv3 license.

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WebTool Presentation

This tool is divided into sections and boxes. The sections are displayed in a black menu, on the left side. There are four sections. The first is the **Home section**, where it is shown a brief presentation of the tool. The second is the **Convergence section**, where it can be uploaded the data and the model adjusted. The third is the **Results section**, where it is displayed graphs, means and probabilistic inferences about the data. The fourth, and last, is the **About section**, where it is displayed information about the authorship of the tool. Inside each section, there are boxes. At these boxes, the users can adjust the information of the graphs and results.

The screenshot displays the BayesBehav webtool interface. On the left is a black sidebar menu with four sections: Home (with a house icon), Convergence (with a gear icon), Results (with a bar chart icon), and About (with a document icon). The main content area is white and currently shows the 'Convergence' section. It contains two adjustable boxes. The first box, titled 'First', has a subtitle 'Insert the family distribution and the number of variables.' and contains two dropdown menus: 'Choose the family distribution' (set to 'Gaussian') and 'How many variables?' (set to 'One'). The second box, titled 'Second', has a subtitle 'Insert your data. The first column is for the factors, the others for the variables. Don't use headings' and contains a 'Choose CSV File' section with a 'Browse...' button and a 'No file selected' status. Two blue callout boxes provide additional context: one on the left points to the sidebar menu, stating 'The section menu of the BayesBehav webtool. Data and Results can be upload and analyzed at the Convergence and Result sections'; another on the right points to the adjustable boxes, stating 'Boxes are used to adjust results and plots'.

The section menu of the BayesBehav webtool. Data and Results can be upload and analyzed at the Convergence and Result sections

Boxes are used to adjust results and plots

Starting the Analysis...

CONVERGENCE

1. First
2. Second
3. Third

First

Insert the family distribution and the number of variables.

Choose the family distribution

Gaussian

How many variables?

One

For starting the analysis, it's necessary to first insert the desired **Family distribution** and the number of **Variables**. It's provided the option of using two probability distributions: Poisson and Gaussian. These two distributions can be suitable to deal with aggressive behavior data. Use the **First** box at the **Convergence** section (on the left).

First

Insert the family distribution and the number of variables.

Choose the family distribution

Gaussian

Gaussian

Poisson

It's provided the option of using two probability distributions: Poisson and Gaussian.

First

Insert the family distribution and the number of variables.

Choose the family distribution

Gaussian

How many variables?

One

One

Two

Three

The number of variables that can be used ranges from one to three.

Starting the Analysis...

CONVERGENCE

1. First
2. Second
3. Third

The **Second** step is to insert your data file in the **Second** box at the **Convergence** section (on the left). The file must be in a CSV format. The data must be organized with the first column for the factors and the others for the variables. **No headlines** should be inserted.

The table shouldn't contain any headings

1	8	17	8
1	4	7	4
1	1	4	1
1	0	1	0
2	44	45	44
2	4	6	4
2	17	25	17
2	0	3	0
3	4	7	4
3	9	10	9
3	2	2	2
3	2	8	2
4	3	6	3
4	5	9	5
4	73	20	73
4	50	27	50
5	7	34	7
5	62	32	62
5	5	10	5
5	61	72	61
6	33	35	33
6	10	17	10
6	21	25	21
6	22	41	22

The first column should contain the factors. These must indicate the time intervals each data belongs. The factors must be represented as numbers or letters (e.g. 1,1,2,2,3,3 or A,A,B,B,C,C).

1	8	17	8
1	4	7	4
1	1	4	1
1	0	1	0
2	44	45	44
2	4	6	4
2	17	25	17
2	0	3	0
3	4	7	4
3	9	10	9
3	2	2	2
3	2	8	2
4	3	6	3
4	5	9	5
4	73	20	73
4	50	27	50
5	7	34	7
5	62	32	62
5	5	10	5
5	61	72	61
6	33	35	33
6	10	17	10
6	21	25	21
6	22	41	22

The other columns should be used for the variables.

Second

Insert your data. The first column is for the factors, the others for the variables. Don't use headings

Choose CSV File

Browse... No file selected

Click on the "Browse" button and search your data file.

Starting the Analysis...

CONVERGENCE

1. First
2. Second
3. Third

The Third box contains five sliders with different functions, but all with the same purpose: Provide a reliable stochastic converged model. This model is achieved when a sequence of essentially random or unpredictable events stabilizes in a pattern. The convergence is achieved by trial and error, updating the model by changing the sliders. Sometimes changing only one slider is enough to achieve convergence (Usually the “n.iter”). Since the model has **converged**, there is **no need** for further updates. The convergence of the model can be checked by the Traceplot and Gelman-Rubin coefficients (**Pages 5 and 6**).

The n.adapt represents the number of adaptations to tune the sampler

The Thin represents thinning rate the algorithm will display to build the models

Third (click to expand)

Adjust your model. (Gelman coefficient should be close to 1)

Number of MCMC iterations

n.iter

1,000 100,000

1,000 10,900 20,800 30,700 40,600 50,500 60,400 70,300 80,200 90,100 100,000

Specify the number of initial samples

n.adapt

1,107 100,000

1,000 10,900 20,800 30,700 40,600 50,500 60,400 70,300 80,200 90,100 100,000

Specify the number of MCMC chains

n.chains

1 2 10

1 2 3 4 5 6 7 8 9 10

Specify the jumps when sampling numbers

Thin

1 5 10

1 2 3 4 5 6 7 8 9 10

The n.iter represents the number of samples to be used on the analysis

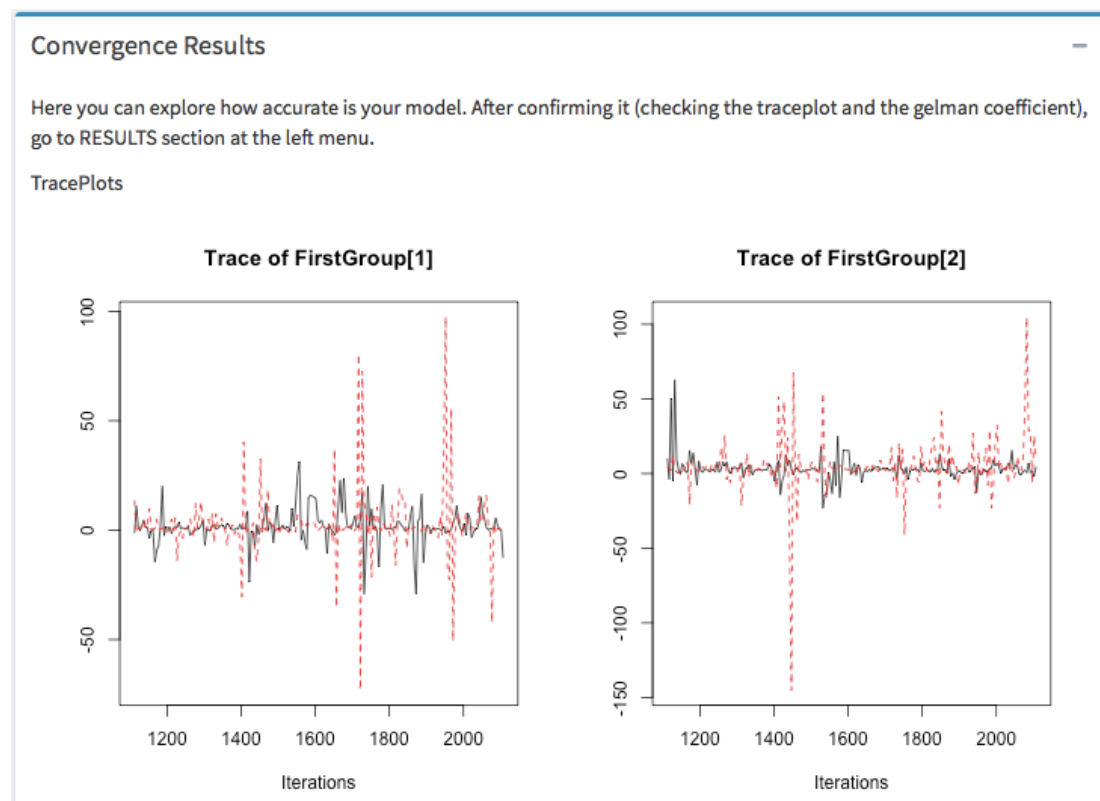
The n.chains represents the number of chains displayed

Starting the Analysis...

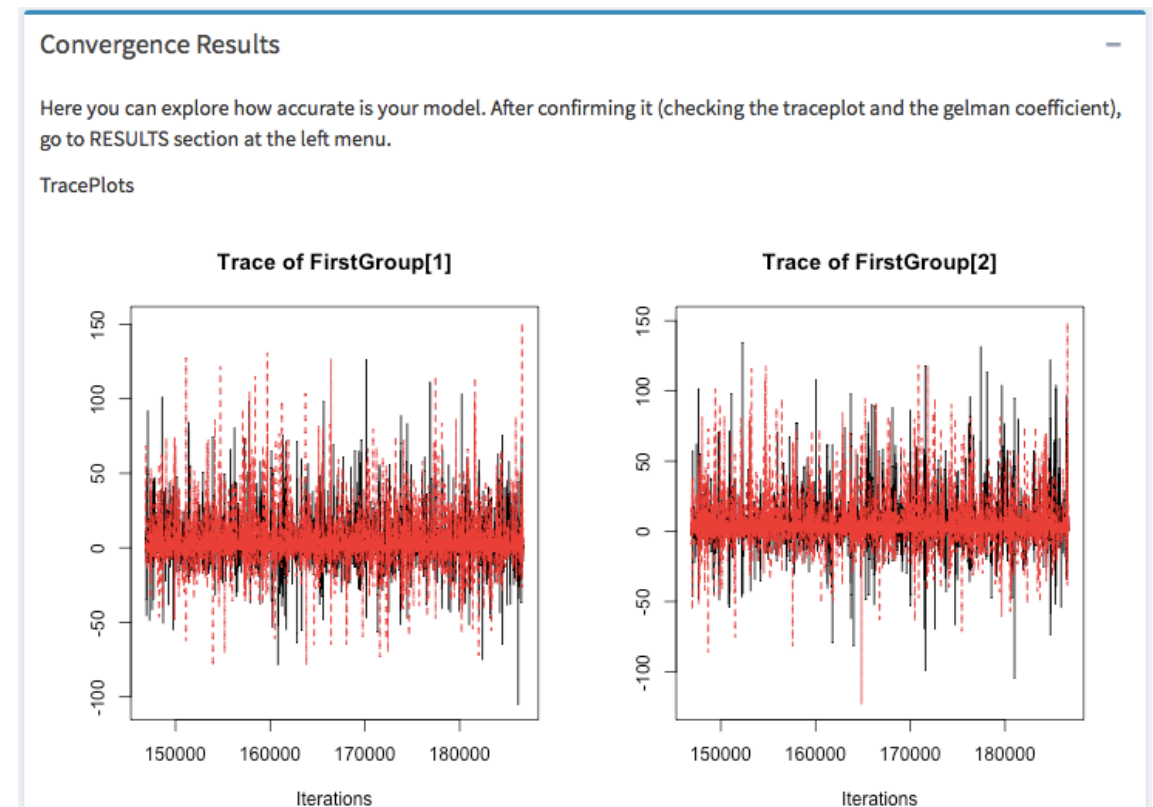
CONVERGENCE

1. First
2. Second
3. Third

The Traceplots is graphical way to visualize the trends from the chains generated. This plot allows identifying different trends, which indicate a non converged result. We illustrate below, two figures. They display a non-converged and a converged traceplot, respectively.



A non-converged Traceplot, displaying a more heterogeneous pattern.



A converged Traceplot, displaying a more homogeneous pattern.

Starting the Analysis...

CONVERGENCE

1. First
2. Second
3. Third

The coefficients of all groups and their Confidence intervals should be close to 1.

The Gelman-Rubin coefficient is a more straightforward way to verify convergence. It provides a coefficient that should be close to **1** when the models are reliable.

Gelman-Rubin Coefficient

Potential scale reduction factors:

	Point est.	Upper C.I.
FirstGroup[1]	1	1
FirstGroup[2]	1	1
FirstGroup[3]	1	1
FirstGroup[4]	1	1
FirstGroup[5]	1	1

Multivariate psrf

1

The Deviance Information Criterion (DIC) is a measure of quality to compare the statistical models. DIC values are inversely related to the compromise between fit and parsimony. It provides three values: The Mean deviance, which correspond to the calculated DIC value; The penalty, which is a correction for the model's complexity; and the Penalized deviance, which is the corrected DIC value according to its penalty

The Penalized deviance is usually the coefficient used to compare models.

DIC Coefficient

Mean deviance: 588.3
penalty 12.04
Penalized deviance: 600.3

Looking the results

RESULTS

1. Plot Inputs

2. Color and Lines

3. Graph Axis

4. Graph Captions

5. Inference Inputs

Plot Inputs

Use the check boxes below to plot distributions at the graph

First Variable

☒ 1° ☒ 2° ☒ 3° ☐ 4° ☐ 5° ☐ 6°

Second Variable

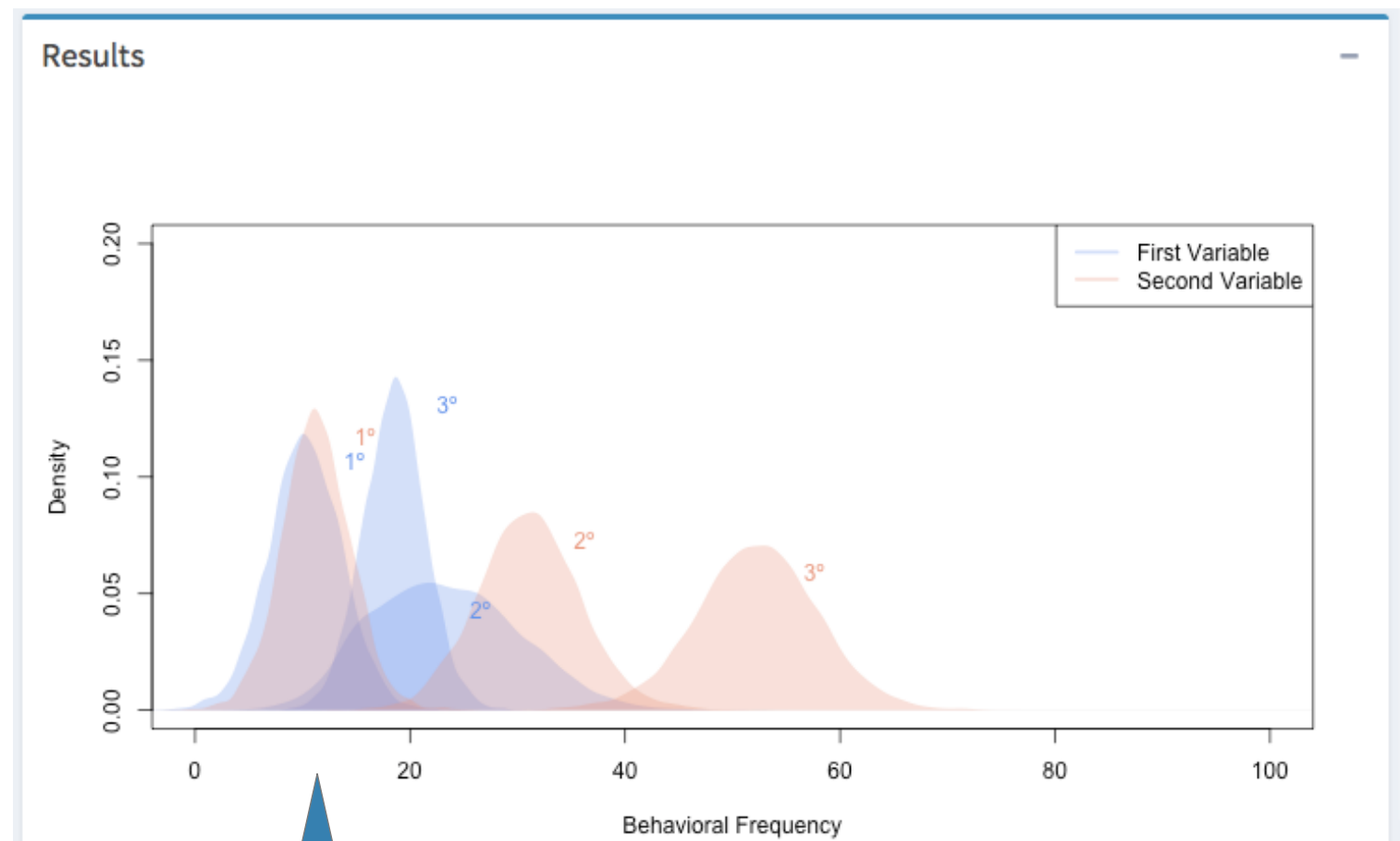
☒ 1° ☒ 2° ☒ 3° ☐ 4° ☐ 5° ☐ 6°

Third Variable

☐ 1° ☐ 2° ☐ 3° ☐ 4° ☐ 5° ☐ 6°

Use the checkboxes to plot distributions on the graph.

The results are displayed in the **Results** section on the left menu. In the **Plot Inputs** box, there are check-boxes for all time intervals of each variable inserted. The desired distributions will be plotted on the graph when clicking on its correspondent check-boxes inputs.



Distributions of different variables can be simultaneously plotted on the graph

Looking the results

RESULTS

1. Plot Inputs

2. Color and Lines

3. Graph Axis

4. Graph Captions

5. Inference Inputs

Plot Inputs

Use the check boxes below to plot distributions at the graph

First Variable

☒ 1° ☒ 2° ☒ 3° ☐ 4° ☐ 5° ☐ 6°

Second Variable

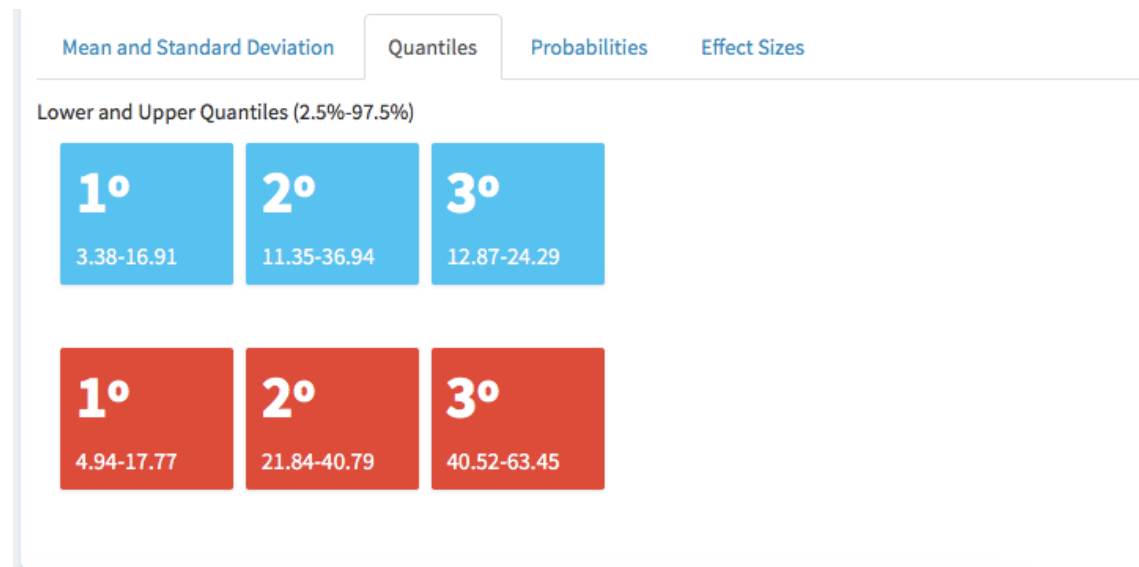
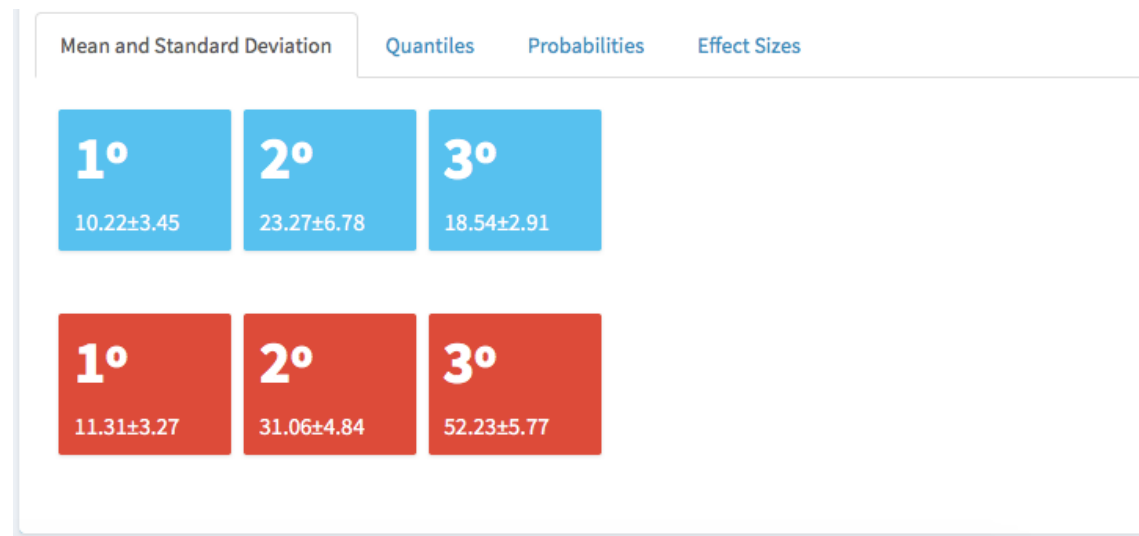
☒ 1° ☒ 2° ☒ 3° ☐ 4° ☐ 5° ☐ 6°

Third Variable

☐ 1° ☐ 2° ☐ 3° ☐ 4° ☐ 5° ☐ 6°

The checkboxes also display the mean and quantile results.

The mean, standard deviations and quantiles will be displayed in the results section when clicking on its correspondent checkbox at the **Plot Inputs** box (on the left).



Looking the results

RESULTS

1. Plot Inputs

2. Color and Lines

3. Graph Axis

4. Graph Captions

5. Inference Inputs

The Color of the distributions can be changed for each of the variables at the **Colors and Lines** box in the **Results** section. In addition, the line's color and type can also be changed.

Color and Lines

First Variable color

Blue

First Variable line color

White

First Variable line type

Solid

Second Variable color

Red

Second Variable line color

White

Second Variable line type

Solid

Third Variable color

Green

Third Variable line color

White

Third Variable line type

Solid

In the inputs, the color of the distribution and its lines can be changed to White, Blue, Red or Green.

The line type can also be changed to Solid, Dashed or Dotted.

Looking the results

RESULTS

1. Plot Inputs
2. Color and Lines
3. **Graph Axis**
4. Graph Captions
5. Inference Inputs

The X and Y axis can be changed using the **Graph Axis** box (on the left).

Use this input for changes
in the X-axis

Graph Axis

X-axis

Y-axis

Use this input for changes
in the Y-axis

Looking the results

RESULTS

1. Plot Inputs
2. Color and Lines
3. Graph Axis
4. Graph Captions
5. Inference Inputs

Graph Captions

- ☒ Insert caption
- ☒ Label the distributions

Name of First Variable

First Variable

Name of Second Variable

Second Variable

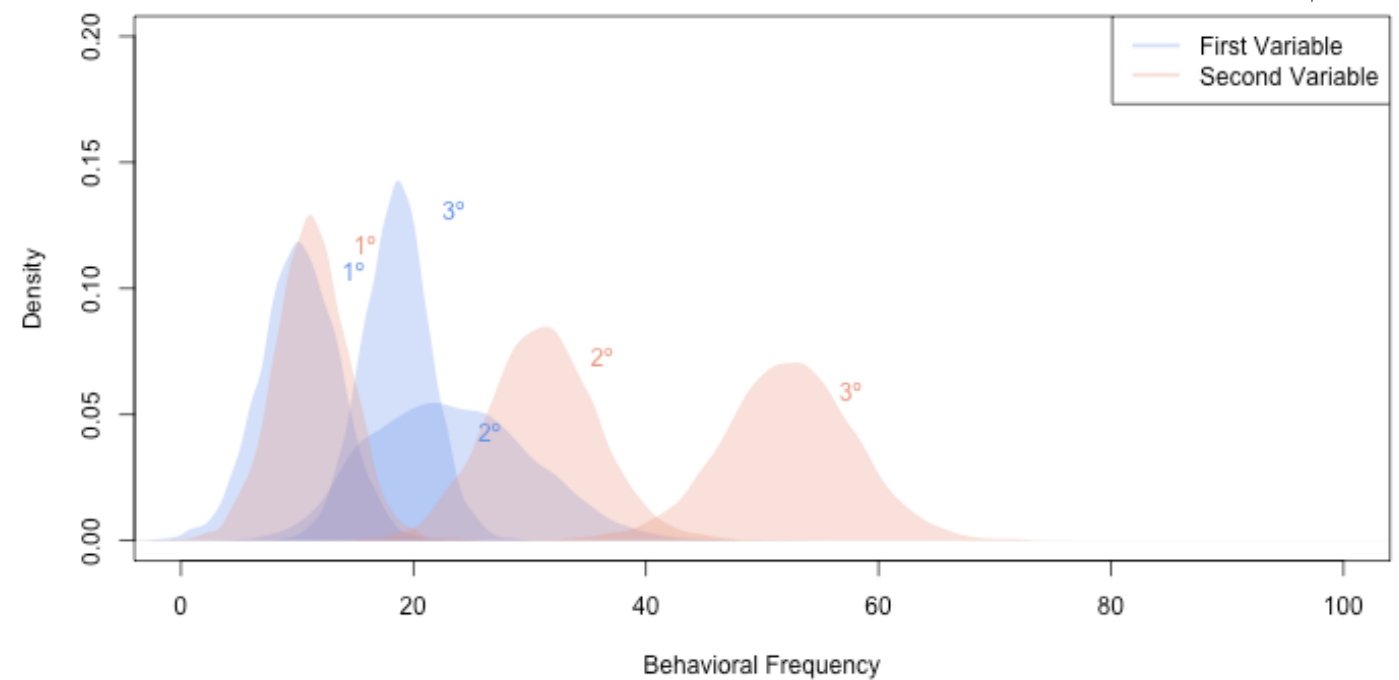
Name of Third Variable

Third Variable

The captions can be inserted and the variables labels can be changed using the **Graph Captions** box (on the left).

Captions can be inserted and labels changed.

Results



Looking the results

RESULTS

1. Plot Inputs
2. Color and Lines
3. Graph Axis
4. Graph Captions
5. Inference Inputs

Comparisons between time intervals of the same variable (**comparison within variables**) and among time intervals of different variables (**comparison among variables**) can be performed. For choosing which distributions to compare, use the sliders at the **Inference Inputs** box (on the left).

The screenshot shows the 'Inference Inputs' panel. It contains two main sections. The first section, 'Comparisons WITHIN variables', has a heading 'Time interval' and a slider ranging from 1 to 8, with a blue square marker at 1. The second section, 'Comparisons AMONG variables', has a heading 'First Variable' and a slider ranging from 1 to 8, with a blue square marker at 1. Below this is a heading 'Second Variable' and another slider ranging from 1 to 8, with a blue square marker at 1. At the bottom is a heading 'Third Variable' and a third slider ranging from 1 to 8, with a blue square marker at 1. All sliders have numerical labels 1 through 8 along the axis.

First and Second sliders are for comparisons within variables

The third, fourth and fifth sliders are for comparisons among variables. These sliders represent distributions of the first, the second and the third variables, respectively.

Looking the results

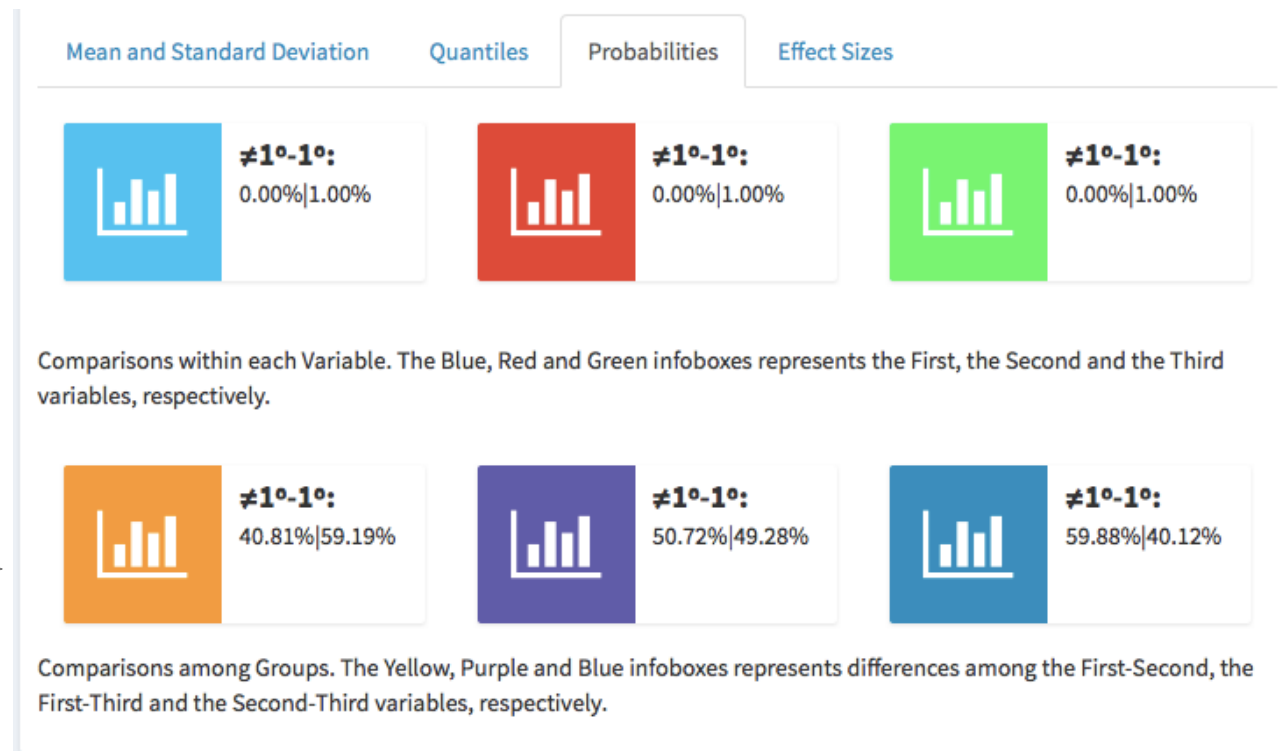
RESULTS

1. Plot Inputs
2. Color and Lines
3. Graph Axis
4. Graph Captions
5. Inference Inputs

Each box displays an inference among **two** time intervals. These time intervals may be from **within** a variable (Light-Blue, Red and Green boxes) or **among** variables (Yellow, Purple or Blue boxes). For choosing which distributions to compare, use the sliders at the **Inference Inputs** box (on the left).

The Blue, Red and Green boxes display the inference results within the First, Second and Third variables, respectively.

The Yellow, Purple and Blue boxes display the inference results among the First-Second, First-Third and Second-Third variables, respectively.



Looking the results

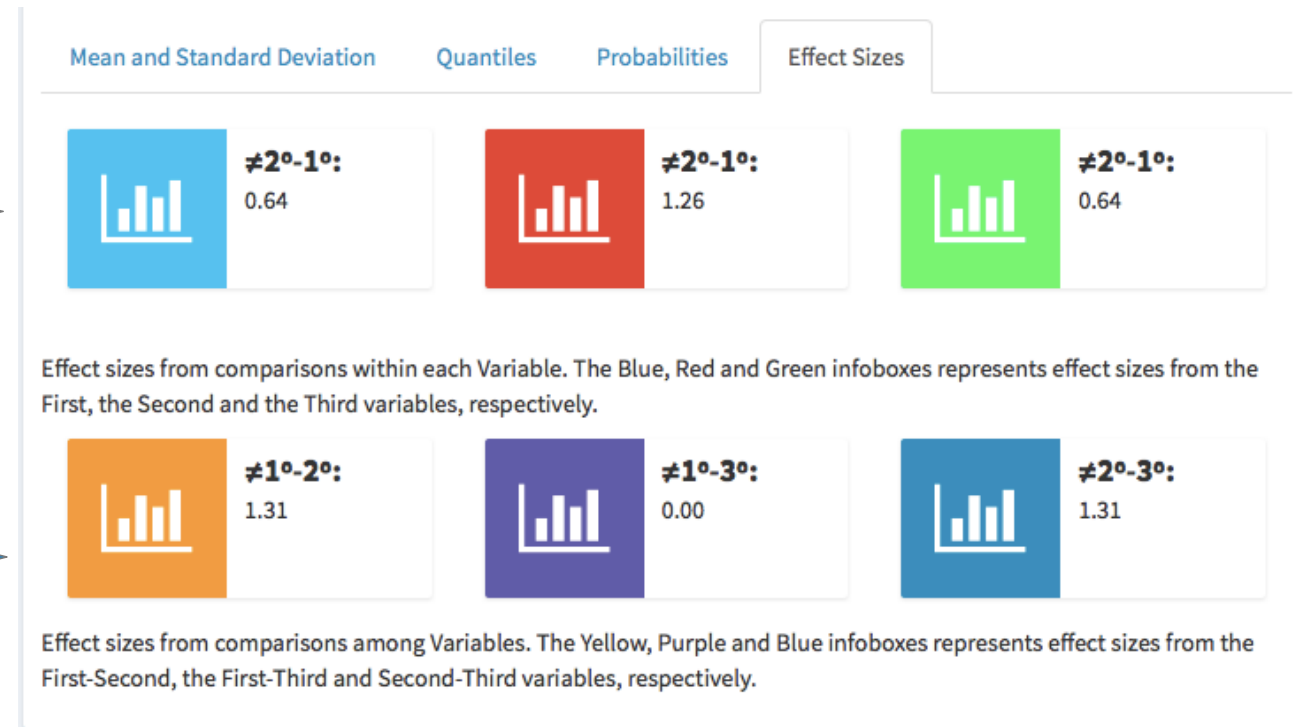
RESULTS

1. Plot Inputs
2. Color and Lines
3. Graph Axis
4. Graph Captions
5. Inference Inputs

BayesBahev also provides the effect sizes from the inference. Effect sizes are important for sample size planning and power analysis. Each box displays an effect-size from the inference of **two** time intervals. These time intervals may be from **within** a variable (Light-Blue, Red and Green boxes) or **among** variables (Yellow, Purple or Blue boxes). For choosing which distributions to compare, use the sliders at the **Inference Inputs** box (on the left).

The Blue, Red and Green boxes display the effect-size from the inference results within the First, Second and Third variables, respectively.

The Yellow, Purple and Blue boxes display the effect-size from the inference results among the First-Second, First-Third and Second-Third variables, respectively.



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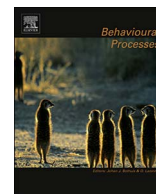
Sections, **5**

Stochastic convergence, **8**

Traceplot, **9**

ANEXO

ARTIGO: "BAYESIAN ANALYSIS IMPROVES EXPERIMENTAL STUDIES ABOUT
TEMPORAL PATTERNING OF AGGRESSION IN FISH."



Bayesian analysis improves experimental studies about temporal patterning of aggression in fish



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ABSTRACT

This study aims to describe a Bayesian Hierarchical Linear Model (HLM) approach for longitudinal designs in fish's experimental aggressive behavior studies as an alternative to classical methods. In particular, we discuss the advantages of Bayesian analysis in dealing with combined variables, non-statistically significant results and required sample size using an experiment of angelfish (*Pterophyllum scalare*) species as case study. Groups of 3 individuals were subjected to daily observations recorded for 10 min during 5 days. The frequencies of attacks, displays and the total attacks (attacks + displays) of each record were modeled using Monte Carlo Markov chains. In addition, a Bayesian HLM was performed for measuring the rate of increase/decrease of the aggressive behavior during the time and to assess the probability of difference among days. Results highlighted that using the combined variable of total attacks could lead to biased conclusions as displays and attacks showed an opposite pattern in the experiment. Moreover, depending of the study, this difference in pattern can happen more clearly or more subtly. Subtle changes cannot be detected when p-values are implemented. On the contrary, Bayesian methods provide a clear description of the changes even when patterns are subtle. Additionally, results showed that the number of replicates (15 or 11) invariant the study conclusions as well that using a small sample size could be more evident within the overlapping days, that includes the social rank stability. Therefore, Bayesian analysis seems to be a richer and an adequate statistical approach for fish's aggressive behavior longitudinal designs.

1. Introduction

In aquaculture, several species of fish are social and interact aggressively to set a dominance hierarchy and for territorial defense. Aggressive behavior can be showed in a very dynamic way, escalating in minutes, hours and days. These dynamic processes can cause injuries and, if continued for long time, lead to individual death, or to increased social stress that, in turn, cause negative effect on growth and general welfare (Damsgård and Huntingford, 2012). Hence, knowledge about the temporal dynamic of aggressive behavior can benefits fish's management, enhancing species growth and welfare (Dow et al., 1976; Gonçalves-de-Freitas et al., 2008; Damsgård and Huntingford, 2012;

Montero et al., 2009). Indeed, bibliography of studies intending to evaluate aggressive behavior in fish is increasingly growing (Gómez-Laplaza and Morgan, 1993; Ros et al., 2006; Almazán-Rueda et al., 2004; Castro and Caballero, 2004; Balzarini et al., 2014). In these articles, ethograms are usually used to understand how fish aggressively interact and to standardize variables patterns for quantifying the number of aggressive responses. Some typical examples of these variables are mouth attacks, lateral threats, and tail beating that can be used individually, partially combined based on its intensity (e.g. attacks and displays), or fully combined in a single one (e.g. total attacks) (Hallen and Wittenberger, 1987; Gomez-Laplaza and Morgan, 1993; Werneyer et al., 2002; Desjardins et al., 2012; Balzarini et al., 2014).

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Combining variables have become a common practice among fish aggressive behavior studies (Gonçalves-de-Freitas et al., 2008; Maan et al., 2001; Ros et al., 2006; Almazán-Rueda et al., 2004; Castro and Caballero, 2004), although there is still no clear the mathematical criteria about when and how do it.

However, several quantitative problems arise when aggressive behavior data are analyzed using both individual and combined variables, because the collection of this information usually presents three main issues: (i) replicates are hard to produce; (ii) the way which these behaviors occur is quite variable within individuals and; (iii) the experimental design is longitudinal (i.e. concerns the same individual over time). The use of classical approaches to account these issues could be problematic as it imposes difficulties in respecting classical parametrical assumptions as normality, homoskedasticity, and sphericity. Additionally, multiple comparisons are usually performed using post-hoc tests (e.g. Terleph, 2004; Carvalho et al., 2012). Nevertheless, when multiple comparisons are implemented, the probability that a researcher wrongly conclude that there is at least one statistically significant effect across a set of tests, increases additively with each test (Gelman et al., 2012). In addition, the evaluation of repeated measures when there are more than one treatment/independent variables and the data are non-parametrical is still a limitation in classical approaches.

The Repeated Measures Analysis of Variance (RM ANOVA) is often used in fish's aggressive behavior studies (e.g. Gómez-Laplaza and Morgan, 1993; Ros et al., 2006; Almazán-Rueda et al., 2004; Castro and Caballero, 2004). Alternatively, the use of Bayesian Hierarchical Linear Models (Bayesian HLM) is grown in popularity highlighting its usefulness in many biological fields including behavioral studies (Alterovitz et al., 2007; McCarthy, 2007; Kinas and Andrade, 2007; McNamara et al., 2006). Bayesian HLMs operate in a multilevel way making comparisons among groups and examining the posterior distributions from different perspectives or margins (Kruschke, 2015). Hence, they are more suitable for dealing with multiple comparison problems, because they shift point estimates and their corresponding intervals closer to each other instead of making confidence intervals wider as in classical estimates corrections (Gelman et al., 2012). Additionally, Bayesian HLMs provide a different view of the probability, defining it as a metric of uncertainty. On the contrary, the classical view of the probability is defined as the limit of the relative frequency of observed data in an experiment if the same procedure of data collection and analysis was implemented multiple times (McCarthy, 2007). For this reason, in a classical approach, we are not able to provide the exact probability of difference between two or more data groups as Bayesian methods do. Classical methods use p-values to access the probability of having an extreme result when the same experiment is repeated multiple times. Many researchers have pointed the limitations of using p-values in these cases as this approach ignores important non-significant results in the conclusions of the studies (Gelman, 2013; Ludwig, 2005; Pitak-Arnnp et al., 2010).

Bayesian analysis can work with resampling procedures, such as Monte Carlo Markov chains, which can numerically approximate Gaussian or different probability distributions (e.g. binomial, Poisson, etc) (Kruschke, 2012). Moreover, Bayesian methods can also implement heteroskedastic robust models that can overcome the assumption of homoskedasticity required in RM ANOVA (Kruschke, 2012), even with small samples sizes (Good and Zellner, 1975; Zellner, 1998). Indeed, as mentioned before, replicates in aggressive behavior studies can be difficult and expensive to be acquired. Consequently, the possibility to reduce sample size without changing the robustness of the conclusions could help scientists dealing with this typical issue.

Considering the importance of aggressive behavior studies for fish's welfare in aquaculture, knowledge on the longitudinal designs of the aggressive behavior is crucial for the proper understanding of aggression in fishes, and its consequences for fish social group as well. Within this context, for the first time, we discuss the advantages of Bayesian analysis in dealing with gathered data, non-statistically significant

results and required sample size. Therefore, this paper aims to test if a Bayesian HLM approach improves modeling longitudinal designs in aggressive behavior study in angelfishes, a social rank organized cichlid fish (e.g. Carvalho et al., 2012).

2. Materials and methods

2.1. Subjects and maintenance

The subject species was the angelfish, *Pterophyllum scalare* Schultze 1823. This fresh water cichlid presents a well known agonistic interaction and social rank organization (Gomez-Laplaza & Morgan 1993). The animals were provided by the Aquaculture Center (CAUNESP) of the São Paulo State University (UNESP) from Brazil. Individuals were kept for 15 days in water tanks of 500L polyethylene (ca. 1 fish/10L), and fed with a commercial ration developed for cichlids, twice a day, in the morning and evening (8:00 and 18:00), until apparent satiety. The water temperature was controlled to 28 °C (Pérez et al., 2003) and its quality was maintained through biological filters (filter 400L/h) and constant aeration. The photophase was kept on 12 h of light (07:00 h to 19:00 h). The tanks were siphoned weekly to remove the remnants of food and feces.

2.2. Experimental design

Fifteen groups consisting of three animals with size between 30 and 50 mm (standard length) were kept for 5 days to behavioral observation. In order to confirm consistency, this experiment was repeated twice. Aggressive behavior was daily recorded during 10 min. The records were run always between 14:00 h and 18:00 h to avoid influence of the circadian rhythm. This interval of time was chosen to avoid the influence of competition for food (Gómez-Laplaza and Morgan, 2003; Grobler and Wood, 2013). The aggressive interactions were quantified by the frequency of aggressive behavior, based on *P. scalare* ethogram (Carvalho et al., 2012). Data was labeled as attacks (more intensive, with contact), displays (less intensive, no contact), and total attacks (attacks + displays) for the analysis (Haller and Wittemberger, 1988; Alvarenga and Volpato, 1995). Body bites, mouth fights and chasing were considered as attacks, while lateral threat, frontal displays and ripples as displays.

2.3. Statistical modeling

Modeling was done with data of attacks, displays and total attacks of each trial that were adjusted with Monte Carlo Markov chains (Metropolis-Hasting algorithm) using non-informative priors (Jeffreys, 1961).

2.3.1. Modeling the rate of increase/decrease

Bayesian regression analysis was performed for measuring the rate of increase/decrease of the aggressive behavior per day (agB/day). The model was specified as:

$$\begin{aligned} W_i &\sim N(\mu_i, \tau_i) \\ \mu_i &= \alpha + bY_i \\ \alpha &\sim N(\sigma, t) \\ b &\sim N(0, 0.10E - 6) \\ \sigma &\sim N(0, 0.10E - 6) \\ t &\sim \text{Gamma}(0.001, 0.001) \end{aligned} \quad (2.1)$$

where W_i indicates the error term for i observations with a normal distribution, σ and t are hyperparameters that specify random variances for the intercept α and slope b of the linear model. Uninformative priors were assigned for all the parameters.

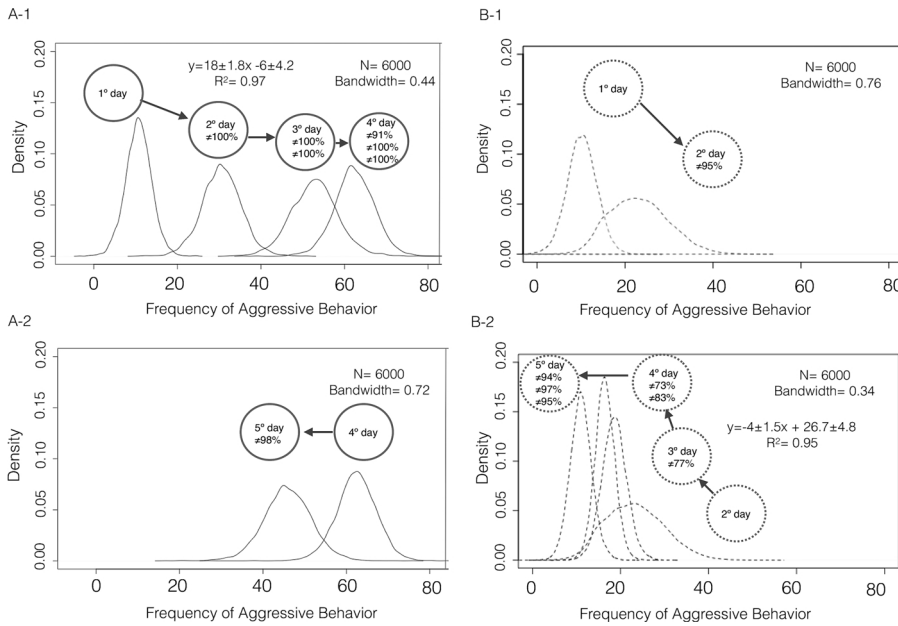


Fig. 1. The daily agB density distributions of the displays (A-1 and 2) and attacks (B-1 and 2) of the first trial. It was observed in 15 min at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

2.3.2. Modeling the probability of difference

A Bayesian HLM was performed to access the probability of difference among days using the last day as the reference class (Wetzels et al., 2012). Subsequently, it was calculated the probability that treatments are equal, i.e. their difference is equal to 0. For this purpose, it was used a boolean variable that counts the number of simulations in which the difference among days are ≥ 0 or < 0 , representing a Bayesian analogue to the classical approach p-value (Gelman et al., 1996; Kéry and Royle, 2016). In this case, the model was specified as:

$$\begin{aligned}
 W_i &\sim N(\mu_i, \tau[Y_i]) \\
 \mu_i &= \alpha + b[Y_i] \\
 b &\sim N(\sigma, t) \\
 \alpha &\sim N(0, 0.10E - 6) \\
 \sigma &\sim N(0, 0.10E - 6) \\
 t &\sim \text{Gamma}(0.001, 0.001) \\
 \tau &\sim \text{Gamma}(0.001, 0.001)
 \end{aligned} \quad (2.2)$$

where W_i indicates the error term for i observations with a normal distribution and b specifies the average for each category Y for the parameter μ . The hyperparameters σ and t indicate random variances for the parameter b and α parameter refers to the intercept (reference class) and is specified by an uninformative prior distribution. As in the precedent model, uninformative priors were assigned for all the parameters.

2.3.3. Reducing sample size

For measuring the feasibility of reducing the sample size, we performed a simulation study, using our data. We used the same model of the probability differences described above. However, we performed it multiple times, randomly taking one replicate away from the data each time. The Cohen d effect size (1988) was calculated to measure the impact of reducing the sample size in data. It was considered a value of 0.2, 0.5 and 0.8 for the small, medium and large effects, respectively (Cohen, 1988).

2.3.4. Plotting the results

A Bayesian model without any reference class parameters was adopted for plotting the results graphically. All the posterior distributions were plotted in parts for didactic purposes.

Here, the model was specified as:

$$\begin{aligned}
 W_i &\sim N(\mu_i, \tau[Y_i]) \\
 \mu_i &= b[Y_i] \\
 b &\sim N(\sigma, t) \\
 \sigma &\sim N(0, 0.10E - 6) \\
 t &\sim \text{Gamma}(0.001, 0.001)
 \end{aligned} \quad (2.3)$$

where W_i indicates the error term for i observations with a normal distribution and b indicates the average for each category Y for the parameter μ . The hyperparameters σ and t specify random variances for the parameter b . Uninformative priors were assigned for all the parameters.

2.4. Software and simulations

Bayesian models were computed using the JAGS software (Just Another Gibbs Sampler) and statistical package “rjags” (Plummer et al., 2006), specifically developed for implement Bayesian methods using the Monte Carlo Markov chains through the R software (R Development Core Team, 2016). The treatments parameters were estimated through simulations. The number of simulations was established by trial and error in the model convergence process. The success or failure in convergence was checked using the criteria of Geweke (Geweke, 1992) and Gelman-Rubin (Gelman and Rubin, 1992). The latter was also used for establishing a period of “burn-in” constituted by the disposal of some chains to help the model convergence.

3. Results

3.1. Rate of increase/decrease and difference probability among days in fish's attacks and displays

For the first trial, the displays increased till the fourth day at a rate of 21 ± 3 agB/day (Fig. 1A-1). This increase was very consistent since the posterior distributions from the first to the third day have not overlapped, being a 100% different from each other. At the fourth day, displays still increased, although this rate dropped to 17.9 ± 1.8 agB/day. This drop caused an overlap between the distribution of the fourth and third day, differing in 91% from each other. At the fifth day, the agB remarkably decreased with a 98% difference from the fourth day (Fig. 1A-2). The attacks increased until the second day (not enough data to calculate a rate) (Fig. 1B-1) and retreated from the second to the fifth day at a rate of -4.1 ± 1.5 agB/day (Fig. 1B-2). Different from

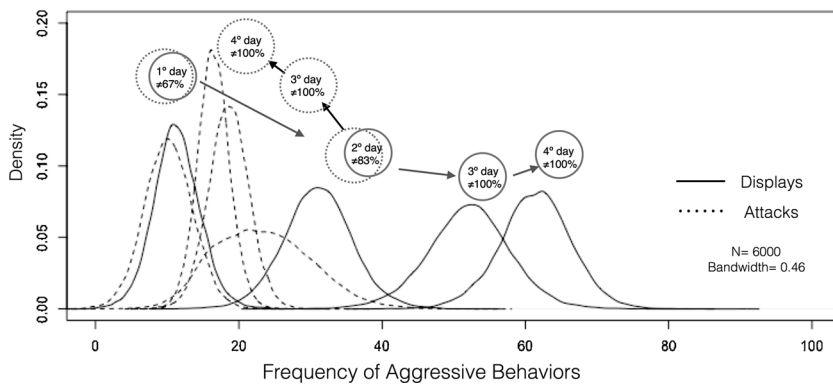


Fig. 2. The daily agB density distributions of the attacks and the displays of the first trial. It was observed in 15 min at each day. They are represented by solid and dashed lines, respectively. Each circle contains the probability of difference between attacks and displays at each day.

Table 1

The mean and 95% credible interval for the standard deviation among and within days of the attacks and displays variables in two trials.

	Attacks		Displays	
	Mean	95% CI	Mean	95% CI
First Trial				
Among all days	13.95	5.27–36.95	30.51	13.02–71.81
Within 1 st day	12	8.15–18.34	7.48	5.05–11.29
Within 2 nd day	21.26	14.54–32.12	24.2	16.65–36.68
Within 3 rd day	12.08	8.23–18.53	32.12	21.85–48.67
Within 4 th day	9.86	6.76–14.67	35.79	24.37–54.37
Within 5 th day	3.07	2.08–4.66	24.47	16.69–37.22
Second Trial				
Among all days	9.49	1.7–28.0	29.85	12.56–72.42
Within 1 st day	13.31	9.0–20.2	12.53	8.58–18.92
Within 2 nd day	27.66	18.6–42.2	18.6	12.74–28.36
Within 3 rd day	11.2	7.6–17.1	22.34	15.18–34.21
Within 4 th day	9.12	6.2–13.8	19.27	13.15–29.24
Within 5 th day	9.63	6.5–14.6	22.91	15.56–34.66

displays, there was a high overlapping of the distributions from the second to the fourth day and an increase in difference at the fifth day. It is noteworthy that the attacks and displays were almost the same till the second day, differing in 83% (Fig. 2). However, at the third day, both behaviors became completely non overlapped till the rest of the study period. In addition, the credible interval for the standard deviation among days more than encompassed the standard deviations within each day (Table 1, Fig. 3A-1 and A-2), meaning that there is more uncertainty in differences among days than within each day for this trial.

For the second trial, the displays increased till the third day at a rate

of 27.9 ± 3.72 agB/day (Fig. 4A-1). The second and third day overlapped in 8%. The displays decreased till the fifth day at a rate of -2.5 ± 5 agB/day (Fig. 4A-2). Although displays decreased at the fourth day, this day was very similar to the third and the fifth ones, differing only in 71% and 54%, respectively. The attacks increased from the first to the second day (not enough data to calculate a rate), decreasing till the fifth day at a rate of -8.6 ± 1.7 agB/day (Fig. 4B-1 and B-2). Different from the first trial, there was a high overlapping only from the second to the third day. At the fourth and fifth day, the decrease was clearer with a slightly overlapping of the distributions on the fourth day and a not overlapping at the fifth day. As in the first trial, the attacks and displays were the same at the first day ($\neq 50\%$) (Fig. 5). Although, at the second trial displays and attacks were more likely to differ on the second day ($\neq 94\%$, compared to 83% in the first trial). Additionally, the rate of increase of displays (till the third day) and a decrease of attacks differed in 92% and 96% between trials, respectively. In addition, the credible interval for the standard deviation among days more than encompassed the standard deviations within each day, in most cases (Table 1, Fig. 3B-1 and B-2). The only exception was the second day of the variable attacks. Therefore, in most cases, there was more uncertainty in differences among days than within each day for this trial.

3.2. Rate of increase/decrease and difference probability among days in fish's total attacks

Overall, the displays were much higher than the attacks. Therefore, they prevailed on the total attacks. Since, attacks retreated and displays increased after the second day, gathering these variables, negatively impacted the rate of increase reducing it from 18 ± 1.8 agB/day to 17.3 ± 3 agB/day at the first trial (Figs. 6A-1 and Fig. 1) and from

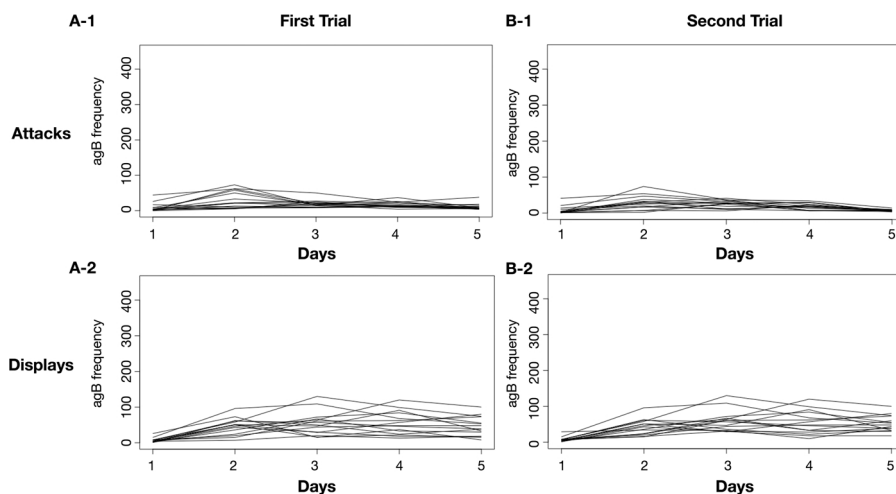


Fig. 3. The daily agB frequency of each of the fifteen groups in five days of observation. It was observed in 15 min at each day.

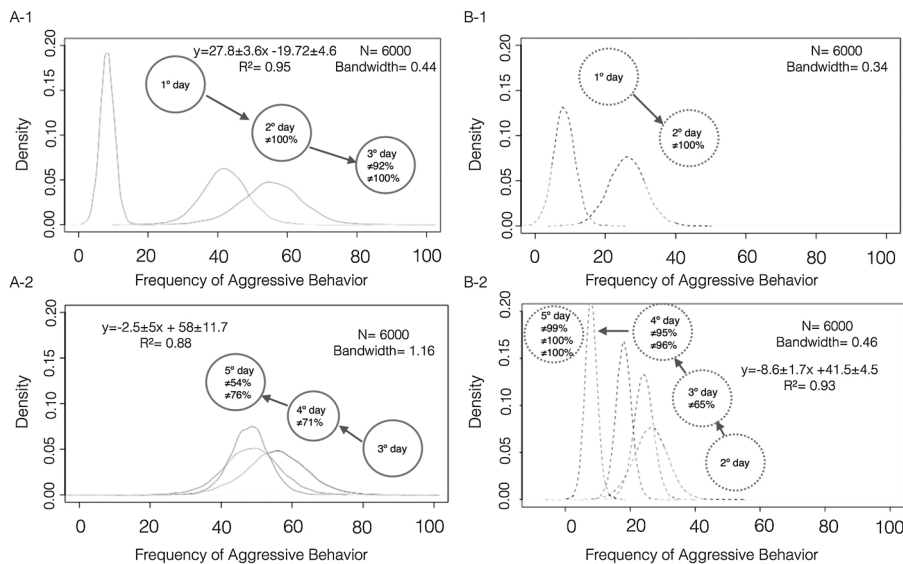


Fig. 4. The daily agB density distributions of the displays (A-1 and 2) and the attacks (B-1 and 2) of the second trial. It was observed in 15 min at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

27.8 ± 3.6 agB/day to 24 ± 8 agB/day at the second (Fig. 6B-1 and Fig. 4). This rate reduction is due to the overlapping of the distributions from the days after the second one, that is the period in which attacks and displays followed different paths (Figs. 2 and 5). Hence, the difference between the second and third day, which is a 100% of displays, dropped to an 84% of total attacks in the first trial. At the second trial the difference of 92% at displays dropped to 79% at total attacks. The rate of increase between total attacks trials differed in 84%.

3.3. Reducing sample size

For displays of the first trials, the reduction of the data sample size tended to decrease the posterior probability of difference among all the days. The unique exception was found between the third and fourth day, in which the posterior probability did not present a clear pattern, also showing a small effect size (Table 2). The difference among the first three days remained the same till the tenth replicate (≠ 100%), and decreasing till the rest of the sample reduction (Fig. 7A-1). Additionally the effect size between these days was considered large (Table 2). For the attacks, the reduction of the data sample size tended to decrease the posterior probability of difference among the first and second day, and the fourth and fifth day. Between these days there was as medium effect size. From the second till the fourth day there was no clear pattern, with a small effect size (Fig. 7A-2 and Table 2).

At the second trial, for displays, the sample size data reduction

tended to decrease the posterior probability of difference among the first three days (Fig. 7B-1). Although, different from the first trial, only the difference among the first and second day remained the same till the seventh replicate (≠ 100%). Between the first and second day there was a very large effect size, while among the second and third days there was a medium effect. For attacks reducing sample size provided similar patterns to the first trial (Fig. 7B-2), although, the first and last two days presented a large effect size instead of a medium one.

4. Discussion

In this study we described how Bayesian analysis could be a reliable alternative to classical methods for longitudinal designs in fish's experimental aggressive behavior studies. Particularly, we presented the advantages of Bayesian analysis in dealing with combined variables, non-statistically significant results and required sample size using as case study an experiment of angelfish (*Pterophyllum scalare*) species.

Our findings revealed how probability degrees, in accordance with the density distributions, provided a clear description of the changes even when patterns are subtle, which cannot be detected using p-values. In addition, Bayesian analysis is enabled to identify whether it is feasible or not to combine variables and reduce sample size. All these subjects are discussed in the topics below.

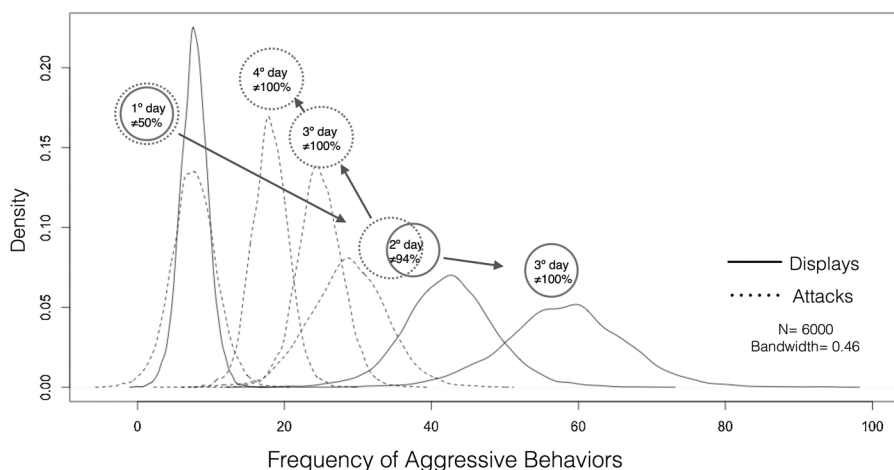


Fig. 5. The daily agB density distributions of the attacks and displays of the second trial. It was observed in 15 min at each day. They are represented by solid and dashed lines, respectively. Each circle contains the probability of difference between attacks and displays at each day.

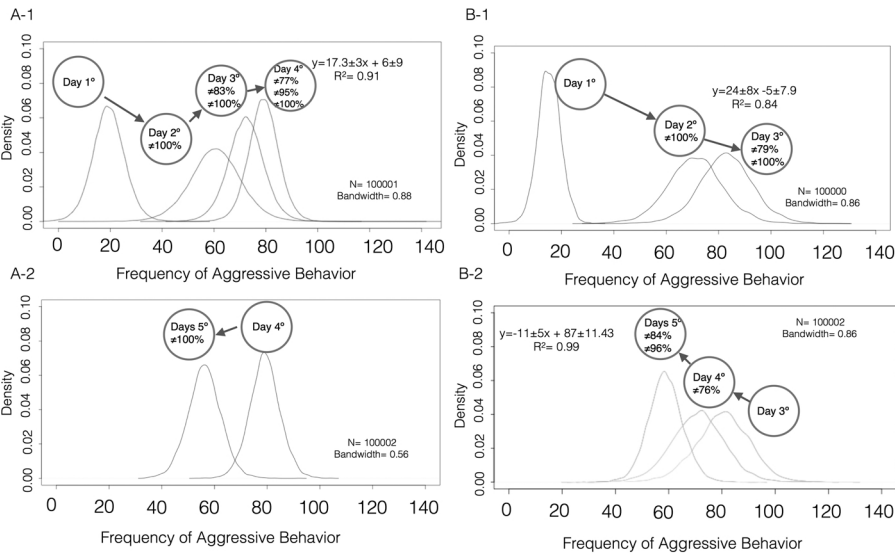


Fig. 6. The daily agB density distributions of the total attacks (TA). A-1 and A-2 represents the first trial. B-1 and B-2 represents the second trial. It was observed in 15 min at each day. Each circle contains the probability of difference from the days before. The first probability is from the first day before, the second is from second day before and so on.

Table 2
Effect sizes between days.

Days	Displays First Trial	Attacks First Trial	Displays Second Trial	Attacks Second Trial
1–2	1.27	0.64	2.00	1.00
2–3	1.00	0.23	0.50	0.12
3–4	0.45	0.20	0.10	0.56
4–5	0.77	0.60	0.60	0.90

4.1. Behavioral consistency

A consistent pattern shows that a specific behavior does not occur by chance. The same experiment, repeated twice, shows a similar pattern for attacks, displays, and total attacks. Gómez-Laplaza and Morgan (1993) have also found similar patterns for *P. scalare*, which reinforce our results. The increase rate of total attacks, attacks, and displays, between the trials, was also similar (differed in 84%, 94%, and 92%, respectively) with the mentioned study. The uncertainty of 8 and 3 agB/day for total attacks, 3 and 3.72 agB/day for displays, and 1.5

and 1.8 agB/day for attacks was small. We can conclude that the rate of increase is almost similar within the replicates of each trial. In addition, the uncertainty of the standard deviation among days was bigger than within days, meaning that most differences are due to time rather than the unknown factors. It is noteworthy, that the second day presented the larger variation within days, for the variable attacks. For the second trial, the variation within this day was even higher than between days. The second day is related to the establishment of the dominance and subordinate relationships within groups, which is related to the increase in high-intensity agonistic behaviors (Ros et al., 2006). There are many known factors which can increase the variability of this social hierarchy, such as size, experience and physiological status (Damsgård and Huntingford, 2012). Hence, this high variation within the second day is probably related to the social rank stability.

4.2. Statistical significance

Aggressive behaviors are subject to changes during the time (Dow et al., 1976). However, these changes can happen so subtly that may not be statistically noticed in a short period of time. For example, in

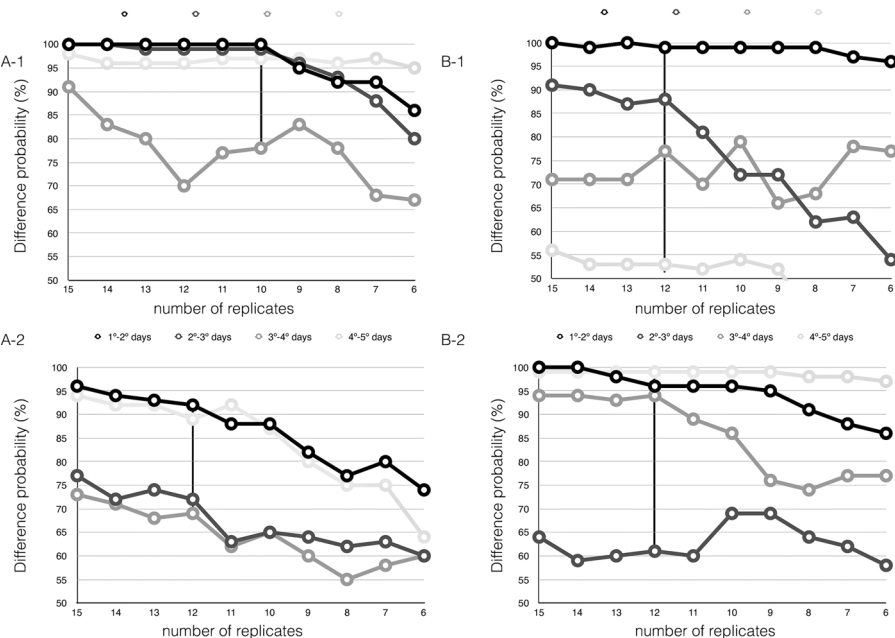


Fig. 7. The distribution of difference between days with different sample sizes. A-1 and 2 represents the displays and attacks of the first trial. B-1 and B-2 represents the displays and attacks of the second trial. The black line is between the first and second day, the dark gray line is between the second and third days, the gray is between the third and fourth days and the light is between the fourth and fifth days. Black line is the cut point.

total attacks the second day is 83% different from third and 95% different from day fourth, but the third day is 77% different from the fourth day (Fig. 6A-1). Using uninformative priors, Bayesian analysis may provide results similar to classical approaches (Jaynes, 1976). When we find 95% of difference, probably we also would find significance with the classical approach ($p < 0.05$). In this case, between days 2 and 3 and 3 and 4 there was no statistical significance considering a 95% credible interval. On the contrary, a statistical difference was found between days 2 and 4. Usually, when this happens, researchers state that the aggressive behavior is the same between the non-statistically significant days even when there is an increase/decrease pattern (e.g. Gómez-Laplaza and Morgan, 1993; Adams et al., 2000; Terleph, 2004; Gonçalves de Freitas and Mariguela, 2006). Indeed when considering p-values we might misinterpret non-significant results (Gelman, 2013; Ludwig, 2005; Pitak-Arnop et al., 2010). Bayesian analysis clarifies the longitudinal changes by building the agB distribution in each day and calculating the probability of difference between them. Therefore, we can measure the strength of the evidence against the null hypothesis (days are equal) using probability degrees (Wagenmakers, 2007). A very intuitive classification was proposed by Raftery (1995) where the categories 50% to 75%, 75% to 95%, 95% to 99% and $> 99\%$ indicates weak, positive, strong and very strong evidence against the null hypothesis, respectively. At the total attacks example, the probability that the third day differs from the second is 83%, and it increases to 95% on the fourth day. Hence, the third day is a transition from second day to fourth day, going from a positive to strong evidence against the null hypothesis. Therefore, the Bayesian approach provides an idea of changes and continuity among days even with the lack of statistical significance, which is more intuitive for a longitudinal design than the equality idea provided by classical methods.

4.3. Combining variables

As the numbers of less intensive aggressive behaviors (displays) were much higher than the numbers of the more intensive ones (attacks), they prevailed on the total attacks, shaping its distribution through time. In the literature, there are many articles using total attacks (e.g. Almazán-Rueda et al., 2004; Wessel et al., 2006; Castro and Caballero, 2004; Earley et al., 2006; Miyai et al., 2011; Carvalho et al., 2012; Pinho-Neto et al., 2014). However, our results highlight that adding attacks to displays diminishes the posterior probability between the days compared to using displays alone, losing their statistical significance considering a 95% credible interval (Figs. 1 A-2 and 5 A-1). This is because attacks and displays are different things in fish's social environment. Attacks (which require more energy) retreat in time when dominance hierarchy is established and displays (requires less energetic cost) increases (Haller and Wittenberger, 1988; Maan et al., 2001; Ros et al., 2006).

This pattern is also described for other species such as juvenile *Oreochromis niloticus* (Alvarenga and Volpato, 1995), *Tilapia zillii* (Neat et al., 1998) and *Oreochromis massambicus* (Ros et al., 2006). Although, this pattern is not clear for all species, as in hatchery-reared steelhead (*Oncorhynchus mykiss*) (Berejikian et al., 2001) and oscar (*Astronotus ocellatus*) (Gonçalves-de-Freitas and Mariguela, 2006). In this sense, whether or not using total attacks may be species-specific and should be previously investigated. In this context, Bayesian analysis can assess the impacts of combining data as attacks and displays by exhibiting the changes in probabilities that happens when using a variable alone and combined. Researchers could use the Bayesian tool to analyze the impact of combining the same variables for other species or other aggressive variables such as bites, chasing or tail beats. Considering the Raftery (1995) rule of thumb any 5% of difference at the posterior probability against the null hypothesis is enough to change from strong (95% to 99%) to positive evidence (75% to 95%). Therefore, we suggest a 5% change in the posterior distribution as a criterion to not

combining data. It is noteworthy that the best procedure will be always to compare the combined variable with the more quantitative single variable (in our example displays). Consequently, the expected effect is always negative or nonexistent since the predominant variable is the main one at the posterior distribution of the gathered variable. The same issue could be differently investigated with classical approaches checking if p-values are > 0.05 or < 0.05 when using the data gathered or not. However, even using this approach the amount of probability change would not be measured. Indeed, if for example, with gathered data the p-value will be 0.01 and with not gathered data of 0.06, this does not mean that using gathered data decreases in 5% the data difference (that is what Bayesian analysis can conclude). On the contrary, it decreases by 5% the probability of having an extreme result if the same experiment is repeated multiple times. Therefore, classical approaches did not measure the impact of combining in our experiment, but the effect of repeating it. Additionally, since classical approaches are mainly focused on p-values and statistical significance, it would not be applied to non-statistically significant results and these can be important in aggressive behavior longitudinal studies as explained in the topic above.

4.4. Reducing sample numbers

The impact of using a small sample size was more evident in days that overlapped more. The inference among these days presented smaller effect sizes. When the effect size of the intervention is large, it is possible to detect such effect in smaller sample numbers, whereas a smaller effect size would require larger sample size (Sullivan and Feinn, 2012). Therefore, attacks were more sensitive to sample numbers from the second till the fourth days, given the smaller effect size and high overlapping among agB distribution. The displays were more sensitive among the third and the fourth day, which are the climax of increase for the first and second trials, respectively. Therefore this sensitivity is due to the uncertainty of the display's increase climax, which can happen at the third or fourth day depending on the individual. Besides the increase in variability, the expected pattern of increase of the agB for displays and decrease for attacks remained robust till the eleventh and tenth replicates, at the first and second trials, respectively. This pattern is related to the social rank stability (Alvarenga and Volpato, 1995; Neat et al., 1998; Ros et al., 2006). After the tenth replicate the variability reaches a level of increase that causes the misleading idea that the agB distribution of some days are equal. Hence, a small sample size could provide the false idea that the agB does not change after dominance hierarchy being established. As previously mentioned, when we rely on p-values with 95% intervals we ignore important no significant information. Thus, the sample size is important to acknowledge the transitional pattern of the agB among days. Therefore a small sample number (≤ 10) can ignore these subtle changes, wrongly transmitting the idea that aggressive behaviors are the same at some days, and even Bayesian estimation can be biased.

The social rank stability is related to this pattern of decrease of the attacks and increase of the displays (Alvarenga and Volpato, 1995; Neat et al., 1998; Ros et al., 2006). Hence a small sample size could provide the false idea that the agB did not change after dominance hierarchy being established.

Usually articles concerning sample size uses power rather than effect size. Effect size and power are related. However, power considers a 95% interval and we are interested at differences that are below this interval. Therefore, we have chosen to work only with the effect size, since it fits better to our proposal.

5. Conclusions

For *P. scalare* the use of total attacks could lead to biased conclusions as displays and attacks showed an opposite pattern in the experiment. The daily changes in aggressiveness happens subtly and

cannot be detected when p-values are implemented. On the contrary, Bayesian methods provide a clear description of the changes even when patterns are subtle. Additionally, results showed that the number of replicates (15 or 11) invariantly the study conclusions as well that using a small sample size could be more evident within the overlapping days, that includes the social rank stability. Therefore, we can conclude that the Bayesian approach could be a richer and an adequate statistical framework for fish's aggressive behavior longitudinal designs.

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Ethical Note

This study was carried out in accordance with the ethical principles of the National Council for the Control of Animal Experimentation (CONCEA, Brazil) and was approved by the Ethics Committee for Animal Experimentation of the São José do Rio Preto campus of São Paulo State University, known locally as the Universidade Estadual Paulista (UNESP), in Brazil (permit number 077/2013). All fish handling was preceded by anesthesia, and substantial efforts were made to minimize animal suffering, as recommended by the Animal Behavior Society's Guidelines.

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