universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

The effect of behavioral experiments on water-borne testosterone level in the neotropical dart-poison frog, *Allobates femoralis*

verfasst von / submitted by

Katharina Dellefont

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 190 445 333
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UF Biologie und Umweltkunde UF Deutsch
Betreut von / Supervisor:	Univ.-Prof. Dott.Leonida Fusani, MPhil PhD

Acknowledgements

This study was funded by the Austrian Science Fund via the project P31518 (PI: Eva Ringler). The experimental protocol was approved by the scientific committee of the "Nouragues Ecological Research Station" and the institutional ethics board of the University of Veterinary Medicine Vienna (ETK10012017), as well as by the Ministry of Science (BMWFW). All handling procedures were conducted in strict accordance with current French and EU law and followed the guidelines of the Association for the Study of Animal Behaviour (ASAB) for the treatment of animals in behavioural research and teaching (ASAB 2019). We thank the Nouragues research field station (managed by CNRS), which benefits from "Investissement d'Avenir" grants managed by the Agence Nationale de la Recherche (AnaEE France ANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). We are grateful to the staff of CNRS Guyane for logistic support in French Guiana. Thanks to Christoph Leeb, Elena Haeler, Sarah Chaloupka and Christian Eliasch for help with fieldwork.

Personal Acknowledgements

I want to thank Prof. Dr. Leonida Fusani who accepted the task of being my supervisor and thus made my work on this project possible. I would like to express my profound thankfulness to Mag. Dr. Eva Ringler who enabled me to work outdoors in the field and guided me through each step of the process, from catching the first frog to handing in the thesis. Likewise, I want to thank both Mag. Dr. Eva Ringler and Mag. Max Ringler, PhD, for the organization of all aspects of the field season including its technical, logistical and financial management. I want to thank Virginie Canoine, PhD, who warmly welcomed and introduced me to the endocrinological lab of Vienna and supported me theoretically and mentally in writing my thesis. Many thanks to my field colleagues, Sarah Chaloupka, who always cared for me as a newbie in fieldwork as well as in the tropical rainforest, and Melissa Peignier, who gave me my first statistical advice. I am thankful for the collaboration of Camilo Rodriguez Lopez, who helped me with my very first hormonal samplings, kept an eye on me during lab work and gave me instructions for RStudio. Additional thanks to Marie-Therese Fischer who always had an open ear and helped me out whenever I was in need. I want to thank Woody Atwood who took care of proofreading my thesis. Finally, I would likewise like to thank Patricia Ullmann and Lena Krische who accompanied me in every stage of this process, as well as my parents who financially supported me and therefore enabled my studies.

Zusammenfassung

„Animal Personality“ Studien bekamen in den letzten Jahrzehnten viel Aufmerksamkeit und stellten zur zentralen Frage, inwieweit unterschiedliche Verhaltens-/ Persönlichkeitstypen auf Unterschiede in der zugrundeliegenden Physiologie, insbesondere hinsichtlich Hormonen, zurückzuführen sind. Hormone spielen eine fundamentale Rolle im Sozialverhalten von Tieren. Insbesondere steht Testosteron in Verbindung mit Veränderungen im reproduktiven Verhalten. Das Ziel dieser Studie war es, Informationen über Variation an Testosteronkonzentrationen innerhalb und zwischen Individuen zu erlangen, sowie den Einfluss vorhergehender Verhaltenstests auf diese Messwerte. Über drei Monate hinweg nahmen wir Hormonmessungen von einer neotropischen Pfeilgiftfroschart, welche in zwei verschiedenen Kontexten durchgeführt wurden. Einerseits nahmen wir sogenannte „Baseline-Messungen“, unabhängig von Verhaltenstests, um individuelle hormonelle Charakteristika einzelner Individuen, sowie kurz- und langfristige Variation der Testosteronkonzentration zu identifizieren. Andererseits wurden Hormonmessungen nach Verhaltensexperimenten - wie in „animal personality“ Studien üblich- genommen. Mit dem Vergleich von Baseline-Testosteronmessungen und Testosteronmessungen nach Verhaltensexperimenten konnte der Zusammenhang zwischen Verhaltenstests und Testosteronwerten ermittelt werden. Ebenfalls versuchten wir Faktoren wie Größe und Alter des Tieres, sowie die Tageszeit zu berücksichtigen, um mögliche Zusammenhänge dieser Faktoren oder Rhythmen (Tages- oder Monatsrhythmus) der Testosteronkonzentration darzulegen. Im Anschluss wurde die intra- und interindividuelle Varianz dieser Messwerte untersucht, um Informationen über die Stabilität der Messwerte, sowie Informationen über individuelle hormonelle Charakteristika, zu bekommen. Die Ergebnisse zeigten, dass Testosteronwerte innerhalb eines Individuums und auch innerhalb der Population relativ konstant bleiben; es gab geringe inter- und intraindividuelle Variation. Die Testosteronwerte wurden durch Verhaltensexperimente moduliert. Bei genauerer Betrachtung der individuellen hormonellen Antwort, zeigte sich jedoch ein zweiseitiges Muster: Bei Individuen mit relativ hohen Ausgangswerten an Testosteron, fanden wir Tendenzen zu einem Abfall der Testosteronkonzentration nach Verhaltenstests, und andererseits einen Anstieg an Testosteron nach Verhaltenstests bei Tieren mit relativ niedrigen Ausgangs-Messwerten. Weiters fanden wir keinen signifikanten Einfluss durch Tageszeit, Alter oder Größe des Tieres auf die gemessene Testosteronkonzentration.

Abstract

Animal Personality Studies has become a frequent field of research in recent decades and focuses on the question of how different behavior and/ or personality types are affected by the individuals´ physiology. Hormones play a fundamental and mediating role in social behaviors of vertebrates. In particular, we focus on testosterone which is associated with changes in reproductive behavior. The goal of the present study was to obtain information about variation of testosterone concentration within and between individuals, as well as to investigate the impact of preceding behavioral testing on testosterone levels. Therefore, we took repeated hormonal measurements in an experimental island population of the dart-poison frog *Allobates femoralis* under two different sets of conditions, over three months. As a control, we conducted independent hormonal baseline measurements of each frog, to determine hormonal characteristics, without any preceding experimental manipulation. In addition, we took hormonal measurements after conducting two common behavioral tests used in animal personality studies and associated with aggressive and exploratory behavior. Through the comparison of the measurements taken at those two different conditions we wanted to determine whether preceding behavioral experiments alter individual testosterone levels. We also gained information about the influence of other factors on testosterone levels, such as temporal variation, as well as age and body size. Our results show that intra- and interindividual testosterone levels are relatively consistent. We found significant differences in testosterone measurements taken independently and immediately after behavioral experiments, with testosterone concentrations generally decreasing after behavioral tests. Time of day, age and body size did not show any significant impact on testosterone measurements. When comparing changes in testosterone measurements (baseline measurements and measurements after behavioral experiments) of individual frogs, we see two tendencies within the population: Individuals with low baseline testosterone concentrations tended to show an increase in testosterone levels after behavioral experiments, while individuals with comparatively high baseline concentrations tended to rather decrease these levels after behavioral experiments.

Introduction

Hormones are essential regulators of social behavior in animals (Velle 1982). Steroid hormones in particular are of great importance in vertebrate reproduction and thus coordinate many social behaviors (Eikenaar et al. 2012). They are well known for the expression of secondary sexual traits, such as behavioral traits (Miles et al. 2007). In the course of this study we focus on testosterone, which is a principal steroid and plays a fundamental role in several social behaviors (Wingfield et al. 2006). For instance, testosterone plays a major role in mediating territorial aggression, courtship and calling behavior in birds (Hirschenhauser, Winkler, and Oliveira 2003; Wingfield et al. 1990; Fusani 2008; Fusani, Beani, and Dessì-Fulgheri 1994), paternal care (through decreased androgen levels) in the Plainfin Midshipman Fish (Knapp, Wingfield, and Bass 1999) and calling activity in anurans (Solís and Penna 1997; Obert 1977). Testosterone has also recently been linked to dominant behavior in humans (Sellers, Mehl, and Josephs 2007).

In nature, levels of testosterone fluctuate, show changes over short-term as well as long-term periods and are affected by different factors (Wingfield et al. 1990). In various species, short-term variations in testosterone levels are caused by social stimuli (Wingfield et al. 1990). For example, the presence of female mating partners or aggressive encounters with conspecifics stimulate testosterone secretion (Ketterson and Nolan 1992). In turn, levels of testosterone also depend on physiological and/or environmental changes, such as age (Kaufman and Vermeulen 2005), time of day (Schlatt et al. 2008; Diver et al. 2003), rainfall, and temperature (Carey and Alexander 2003). Further, the increase of testosterone can be activated by certain life cycle stages, e.g. to prepare the individual for territorial defense, courtship and mating, or breeding (Soares et al. 2010). As persistent high levels of testosterone are energetically costly (Wingfield, Lynn, and Soma 2001; Apfelbeck et al. 2013) and may even reduce the immune function (Miles et al. 2007), such short and long term fluctuations help to optimize the energetic costs.

"Animal Personality" represents a new field of research that receives increasing attention. The concept "personality" offers a new perspective on animal behavior and refers to the observation, that behavioral tendencies often differ between individual animals of a species and/or population and show within-individual consistency across time and/or conditions (Careau et al. 2008; Dall, Houston, and McNamara 2004). Although intra-individual consistent differences, in one or more personality traits (e.g. aggressiveness, exploration, boldness), have been omnipresent in the past (Careau et al. 2008), they were rather seen as variation around the population mean (Carere and Locurto 2011). When analyzing behavior within an animal

population, differences between individuals were not specifically taken into account. However, there is increasing evidence that these inter-individual differences in behavior are highly relevant in ecological and evolutionary terms. As they impact on an animal's prospects of survival, competitive ability, finding mates and other essential resources (Wingfield et al. 2006; Dingemanse and Réale 2005; Sih, Bell, and Johnson 2004), animal personalities are directly linked to individual fitness.

In various animal taxa levels of individual aggression are highly repeatable; some show constantly higher levels of aggression and others constantly lower (Sih, Bell, and Johnson 2004). Since aggressive behavior is commonly linked to testosterone (Hirschenhauser, Winkler, and Oliveira 2003; Wingfield et al. 1990), the question arises, to what extent differences in behavioral phenotypes can be attributed to overall endocrine differences, particularly testosterone.

In this study our question focuses on the inter- and intra-individual consistency of testosterone measurements, to gain information about the existence of individual hormonal characters and to show whether behavioral experiments modulate testosterone concentration. To do so, we compared repeatedly taken testosterone measurements before and after behavioral tests. The behavioral tests, conducted as commonly used in animal personality studies, relate to territorial and explorative behavior. We used as a model the Neotropical poison frog *A. femoralis*, a highly territorial species (Duellman and Trueb 1994; Ringler, Ursprung, and Hödl 2009) that shows highly aggressive behavioral response towards acoustic playbacks, simulating calling intruders. Recently, a non-invasive water bath method (Baugh et al. 2018; Rodriguez et. al in prep.) has been developed, which enabled us to take repeated testosterone measurements of the same individual in the field. Our experimental design allowed us to answer following questions:

(1) Are testosterone levels consistent within and across individual *A. femoralis* males?
(2) Does testosterone level undergo a diurnal rhythm?
(3) Do age and body size affect concentrations of testosterone in *A. femoralis* males?
(4) Does a preceding behavioral test affect individual testosterone levels?

Materials and methods

Study site and study species

Fieldwork took place in a lowland rainforest, close to the field camp 'Saut Pararé' (4°02′ N, 52°41′ W) in the nature reserve 'Les Nouragues' (http://www.nouragues.fr/), in French Guiana (Bongers et al. 2001). Our study area is an island surrounded by the Arataye river and has a size of approximately 5 ha (Ringler et al. 2016). We conducted the fieldwork during the rainy season, from the beginning of February 2019 until the end of April 2019, which coincides with the reproductive season of the studied species (Montanarin, Kaefer, and Lima 2011; Gottsberger and Gruber 2004). The island population of *A. femoralis* was established in 2012 by introducing tadpoles from the nearby mainland population. Previously, there were no *A. femoralis* on the island (Ringler et al. 2014).

Allobates femoralis (Boulenger 1883) is a small, diurnal poison frog (Dendrobatidae), which is distributed throughout the Amazon Basin and Guiana Shield, and occurs in local disjunct populations (Amézquita et al. 2009). In this species, males are highly territorial and advertise territory occupancy by producing loud advertisement calls from exposed, elevated positions (Duellman and Trueb 1994; Weygoldt 1987; Ringler, Ursprung, and Hödl 2009) to female mating partners and male competitors (Roithmair 1992; Narins, Hödl, and Grabul 2003; Ringler et al. 2011). Calling conspecifics that enter a territory get physically attacked by the territory holder (Narins, Hödl, and Grabul 2003). Females show site fidelity and have not been observed responding with aggressive behavior to approaching males or females (Ringler, Ursprung, and Hödl 2009). Females actively approach neighboring calling males for courtship and mating (Ringler et al. 2012; Stückler et al. 2019), which take place inside male territories (Roithmair 1992; Ringler et al. 2012; Montanarin, Kaefer, and Lima 2011). After the eggs hatch into tadpoles, it is the male´s duty to carry them on its back to aquatic sites outside the territory (Ringler et al. 2013).

General population monitoring

We monitored the population from the beginning of February until the end of April, with the intention of gaining information about spatial distribution and changes or stability of individual territory. For this reason, the island was systematically examined throughout the whole field period. We caught the frogs with transparent plastic bags and determined their sex by the existence of the vocal sac (male-presence of vocal sac, female-absence of vocal sac). To record the precise locations of the frogs on a digital map (Ringler et al. 2014), we used a tablet PC

(WinTab 9, Odys, Willich, Germany) equipped with the mobile GIS software ArcPad 10.2 (ESRI, Redlands, CA, U.S.A.). We took pictures of the lateral sides and the ventral and dorsal side of all frogs in front of a measurement grid, which we used for the identification and measurement of individuals. We compared the belly pictures in the field to ensure the identification of each frog (Ringler, Mangione, and Ringler 2015) and later verified it with the pattern matching software Wild-ID (Bolger et al. 2012).

Experimental design

To gain information about the inter- and intra-individual consistency in levels of testosterone and further to investigate the effect of preceding behavioral tests on hormonal measurements, we conducted the following experiment (figure 3).

Experimental procedure:

Since *A. femoralis* is a diurnal species, we always sampled the frogs from 8 to 12:30 am and 2 to 7 pm, which corresponds to their active hours. Due to elevated temperatures around noon, frogs do not show high activity, and therefore no measurements were taken during that time. The fieldwork consisted of two parts, taking hormonal samples and conducting behavioral experiments.

We repeatedly took testosterone measurements under two different conditions:

1) To answer the question of how variable/consistent testosterone levels are within and across individual *A. femoralis* males, we conducted 'baseline measurements' (H0), which refer to the sampling of hormones completely independent from behavioral tests. To this end, the frogs were captured as quickly as possible without the help of any acoustic stimuli (e.g. loudspeaker) and immediately transferred to the water bath (= hormonal sampling, for further explanations see paragraph: Hormonal sampling).

2) To investigate the extent to which behavioral experiments influence circulating levels of testosterone, we additionally took testosterone measurements immediately after behavioral experiments (H1), referring to aggressive and explorative behavior (further details in following paragraph: Behavioral experiments) and compared those with the "baseline measurements", to investigate how stable those measures are across experimental contexts.

Additionally, in every trial we noted the time of day and date when the measurement was taken, as well as individual parameters such as age and body size, to investigate possible other correlates of individual testosterone levels.

We aimed to test each frog in three experimental cycles (see figure 1), each consisting two (H0/ H1) hormonal measurements. Within each cycle there was a time gap of at least 24 hours between H0 and H1 measurements, and between cycles there was a minimum gap of 3 days to assure reliable measurements. Half of the frogs were tested with H0 first, and the other half with H1, and subsequent tests always alternated between conditions. We aimed at obtaining 6 measurements (3 H0, 3 H1) per individual male frog.

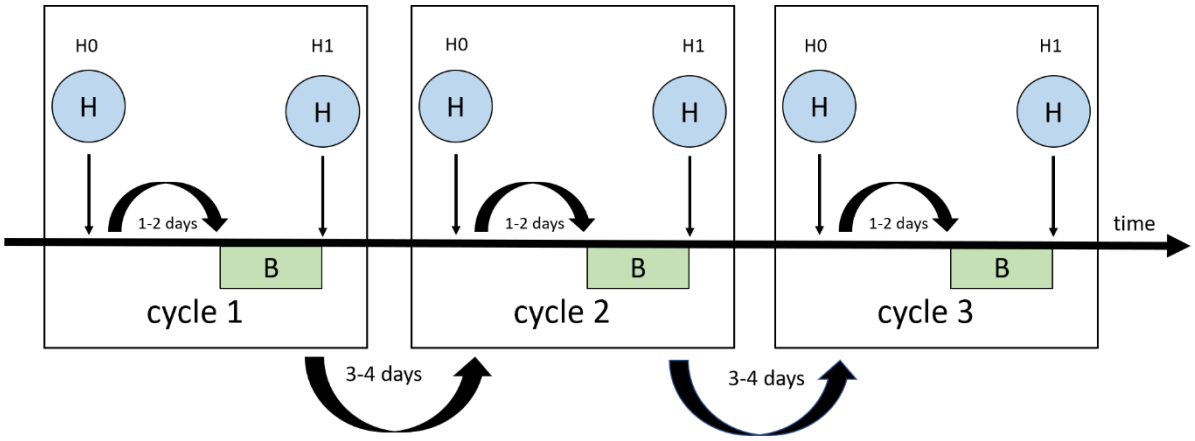


Figure 1: Experimental design. 3 cycles, H = hormonal sampling, B = behavioral experiment (aggression and exploration tests), each cycle 2 measurements (H0/ H1), H0: hormonal sampling without behavioral experiments before/ baseline measurements, H1: B+H, behavioral experiment (aggression and exploration tests) followed by hormonal sampling**.**

Behavioral experiments

In the course of a parallel ongoing project investigating the interplay between sexual selection and animal personality, we conducted a series of behavioral experiments to gain information about individual variation and consistency in aggressiveness, boldness and exploratory tendencies in our model species.

Simulated territorial intrusion:

First, we quantified levels of territorial aggression in individual males by simulating a calling intruder inside a male's territory, via a synthetic conspecific call broadcast by a loudspeaker at a minimum of 66 dB (figure 2). We started the playback and noted the subsequent behavior of the focal male, from the first head body orientation and first jump towards the speaker, until the frog reached a 40 cm perimeter around the loudspeaker. The temporal sequence of behaviors was documented with the help of a voice recorder. From these recordings we extracted the

latency of the first head-body reaction, the latency of the first jump and the speed of the approach (time between first jump and first contact with the perimeter).

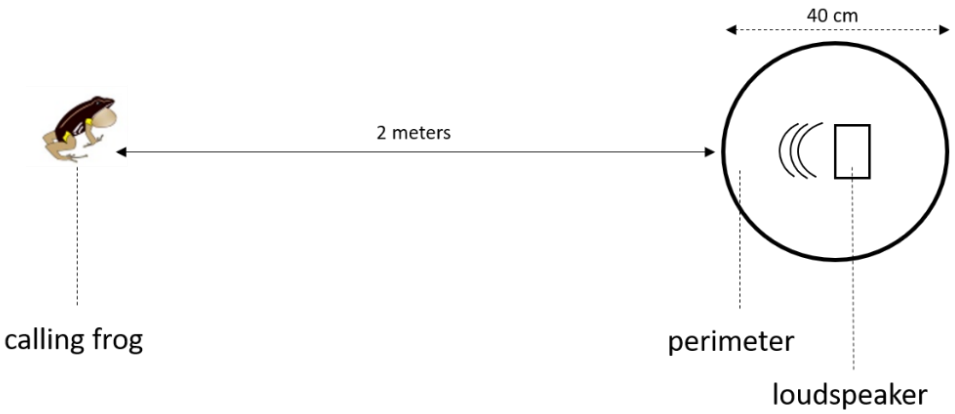


Figure 2: Behavioral experiment – Aggression trial

Exploration test:

Immediately after the aggression test, we caught the frog and conducted the exploration test. For this, we first put the frog in a shelter for five minutes, to allow the frog to recover after handling. Afterwards, we opened the shelter and allowed the frogs to enter the experimental arena (figure 3). A camera recorded the movements of each frog for 15 minutes. From those video recordings we extracted following information: whether the frog exited the shelter (yes/ no), time it took getting out of the shelter, number of jumps, number of jumps on walls, number of jumps on top of obstacles, distanced travelled in box. Immediately after those 15 minutes, frogs were either released at their original location or put into a water bath for hormonal sampling. Time from the beginning of the behavioral experiments until the frog was sampled was approximately 30 minutes.

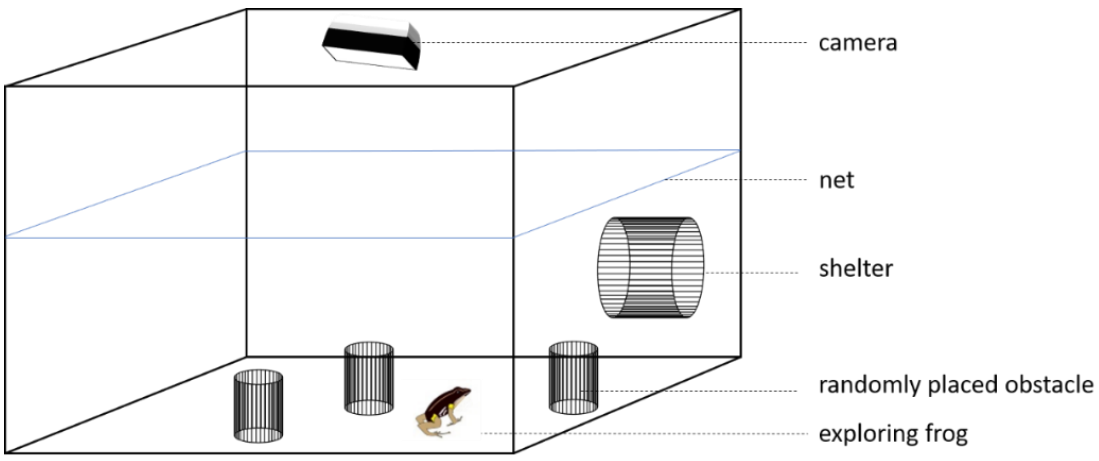


Figure 3: Behavioral Experiment – Exploration box

Hormonal sampling

The hormonal sampling was carried out using a non-invasive water-bath method (Baugh et al. 2018). This method allowed us to obtain repeated hormonal measurements of the same individual. After capture, frogs were put in a glass box, filled with 40 ml of distilled water for one hour. The water covered the bottom of the glass box, so that at least 2/3 of the frogs´ body was outside the water. Additionally, the box was big enough that the frog could easily move. To collect non-polar hormones out of the water, each sample was extracted with C18 cartridges (Phenomenex, #8B-S100-SBJ) with an estimated flow rate of 10 mL/min. Afterwards samples were eluted with 4 ml of absolute ethanol (Baugh et al. 2018) and collected it into glass tubes. Subsequently, the samples were stored in a refrigerator (4 °C) for further processing in the endocrinological Lab of the University of Vienna.

Hormonal analysis:

In order to quantify the mean testosterone concentrations of the samples we used a commercially available ELISA kit, Enzo Life science #ADI-900 065. This kit is suitable for our questions, since it has a very low cross-reaction with other androgens and steroids.

In general, before proceeding with the ELISA, eluted EtOH- samples need to be completely dried down and then re-suspended with 250 ul Assay Buffer (provided by the ENZO Life science kit). However, we first wanted to figure out if 1 ml out of the 4 ml EtOH-samples were sufficient to detect testosterone, in order to spare the rest of the samples for future hormone analyses. Several tests were conducted, which confirmed that 1 ml was sufficient for reliable hormonal measurements. Therefore, in this study 1 ml out of 4 ml EtOH-eluted samples was dried down under a N2-stream at 37 degrees (Condensator, Stuart) and resuspended in 250 ul Assay Buffer. ELISA (Enzyme Linked Immunosorbent Assay) is a plate-based assay technique, to quantitatively determine testosterone in biological fluids, making use of on antibody-antigen reaction. Antibodies are immobilized and attached to the bottom surface of each well. Sample antigens and added enzyme-linked antigens compete for antibody binding sites. Those enzymes interact with an added substrate, to enable color development. Hence the color can be read with a photometer at 405nm. The intensity of the color is inversely proportional to testosterone concentrations, and therefore measured optical densities are used to calculate the concentrations. Concentrations of samples were then corrected for dilution effects. The intra-assay variation % CV´s of all samples was below 5,285.

Statistical Analysis

The statistical analysis was conducted in RStudio (RStudio Team, 2015). All variables were tested for normality using the "Shapiro-Wilk normality test". When data showed a significant deviation from a normal distribution, we applied Wilcoxon signed-rank test. Since the variable "concentration" significantly deviated from a normal distribution (W = 0.84, p-value = <0.001), it was log-transformed (concentration log: W = 0.99, p-value = 0.07). To investigate factors that significantly affect testosterone concentration, we fitted a linear mixed model using the function "lmer", in the package "lme4", with condition (H0/H1), time of day, age, body size and trial order as fixed factors, the individual as a random factor, and concentration (log transformed) as the response variable. In order to enhance model fit we also z-transformed the fixed factors. We verified that the assumptions of the models were not violated by diagnostic plots and normality tests of the residuals. From the linear mixed model, we inferred the repeatability of the testosterone measurements, using both the full (H0 and H1) and reduced (H0 only) datasets, by calculating the Intraclass Correlation Coefficient (ICC= individual variance/ (individual variance + residual variance). To test for differences within individuals across conditions, we used a paired design.

Results

We examined 41 male frogs, of which 33 fully completed three cycles of the experimental design (fig. 3). The remaining frogs (N = 8) either disappeared or died before all measurements could be taken. We examined 115 samples of individuals that were first sampled in the year 2019 and thus considered first-year adults; 137 samples were taken from frogs we already knew from the year 2018 or earlier and thus defined as adult survivors from previous seasons. Since we took pictures of the frogs on a measurement grid to ensure the calculation of the body size, we computed a mean body size of 2.905 cm. The smallest individual had a size of 2.663 cm and the largest 3.213 cm.

We collected a total amount of 252 hormonal samples, where 130 were baseline measurements and 122 were examined after behavioral tests. The baseline concentration showed ranges from 64.52 pg/ml to 1675.6 pg/ml with an average of 397.23 pg/ml (SD = 249.61). The H1 measurements exhibited ranges from 61.82 pg/ml to 1303pg/ml, with an average of 305.08 pg/ml (SD = 163.14). The results of the linear model revealed that condition (H0/H1) was the only significant predictor for testosterone concentration (Chi^2 = 11.66, df = 1,

p = 0.0006, see table 1, figures 4-7). On average, levels of testosterone were lower within individuals after the behavioral tests, compared to the baseline condition (Wilcoxon signed rank test, W = 528, p-value = 0.0003, figure 4). All other factors did not show significant impact (p > 0,3, table 1, figures 5,6,7). The measurements of testosterone were repeatable, in both the full (R = 0.33) and the reduced (H0 measurements only) dataset (R = 0.32), and the variation between individuals was twice as high (0.2185) as the variation within individuals (0.1039).

factor	Chi^2	df	p
H0/H1	19.2136	1	>0.001
body size	0.0154	1	0.90127
age	0.5682	1	0.45099
am/pm	3.2149	1	0.07297

Table 1: The significant difference in testosterone concentrations between the two different conditions (H0 = independent baseline measurements, H1 = measurements after behavioral test), is shown with this table and the following plot (figure 4). Circulating levels of testosterone were significantly lower after behavioral tests than baseline measurements. All other factors had no influence on testosterone concentrations.

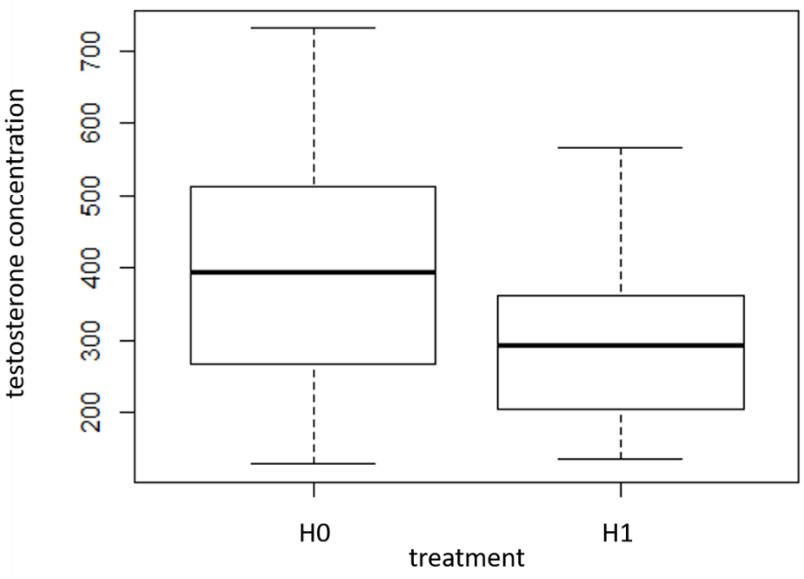


Figure 4: Boxplots of all testosterone concentrations of H0 and H1 measurements. There is a significant difference between the condition/ treatment H0 and H1 (p = 0.0003). Measurements after behavioral tests (H1) are lower than baseline measurements (H0).

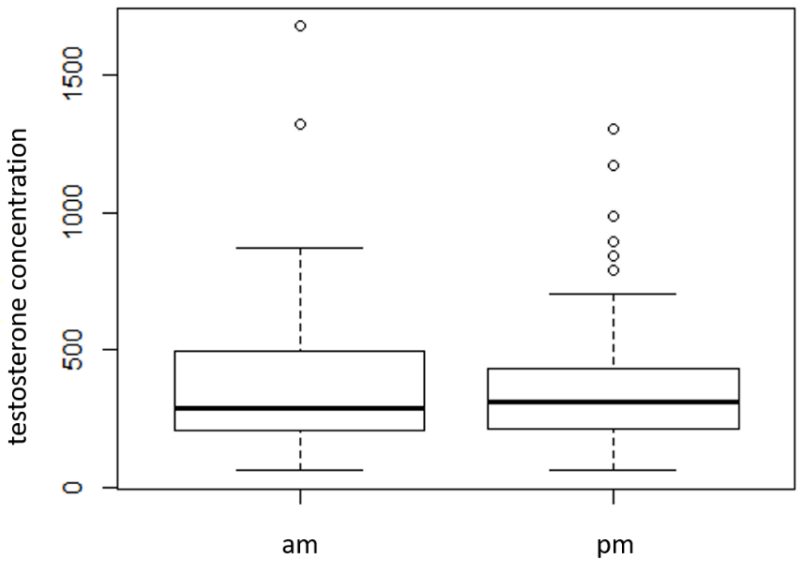


Figure 5: Box plot showing the differences in testosterone measurements taken in the morning or in the afternoon. There was no significant effect of daytime on testosterone concentrations.

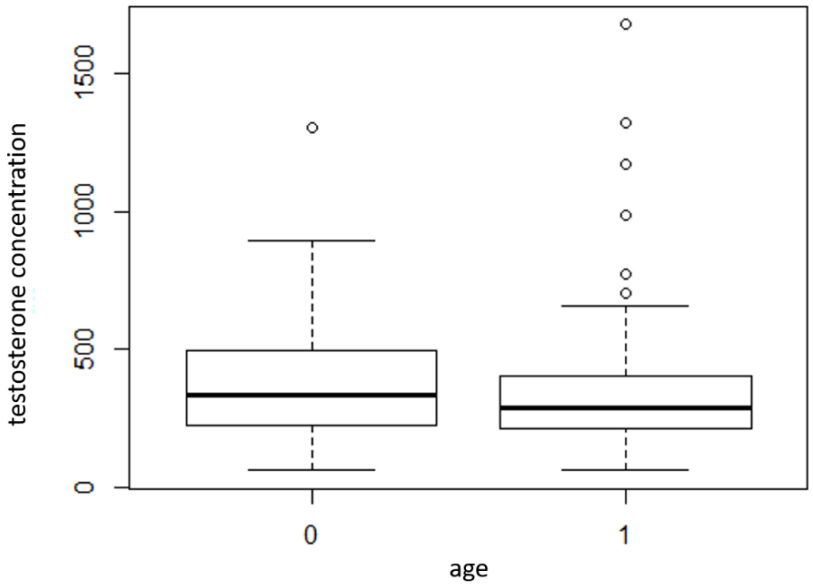


Figure 6: Differences in testosterone concentrations between 0 = first year adults and 1 = survivors from previous seasons. This comparison did not show any impact of age on testosterone levels.

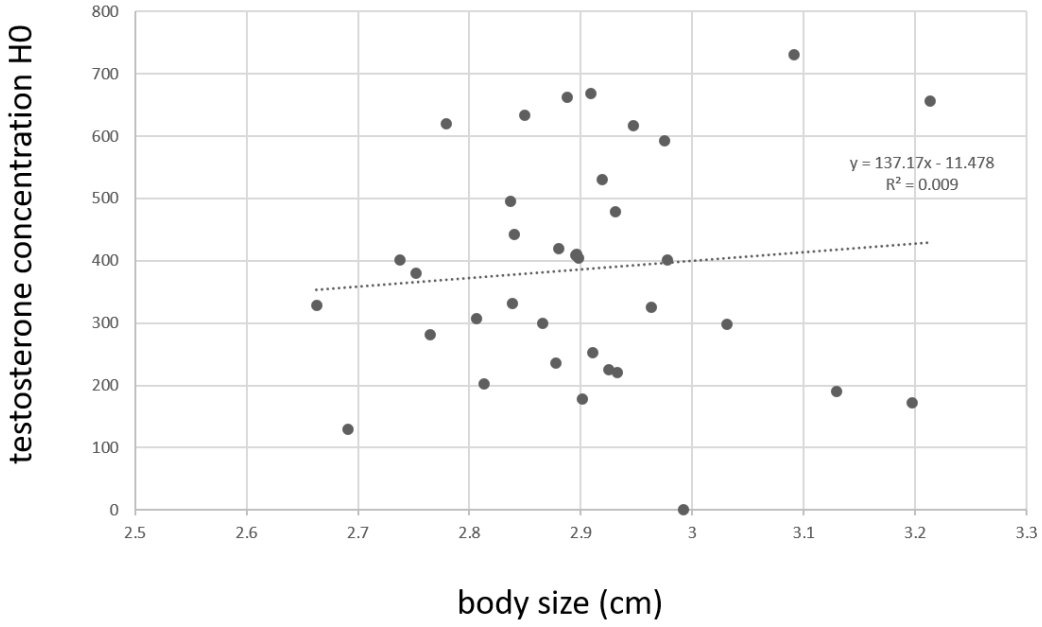
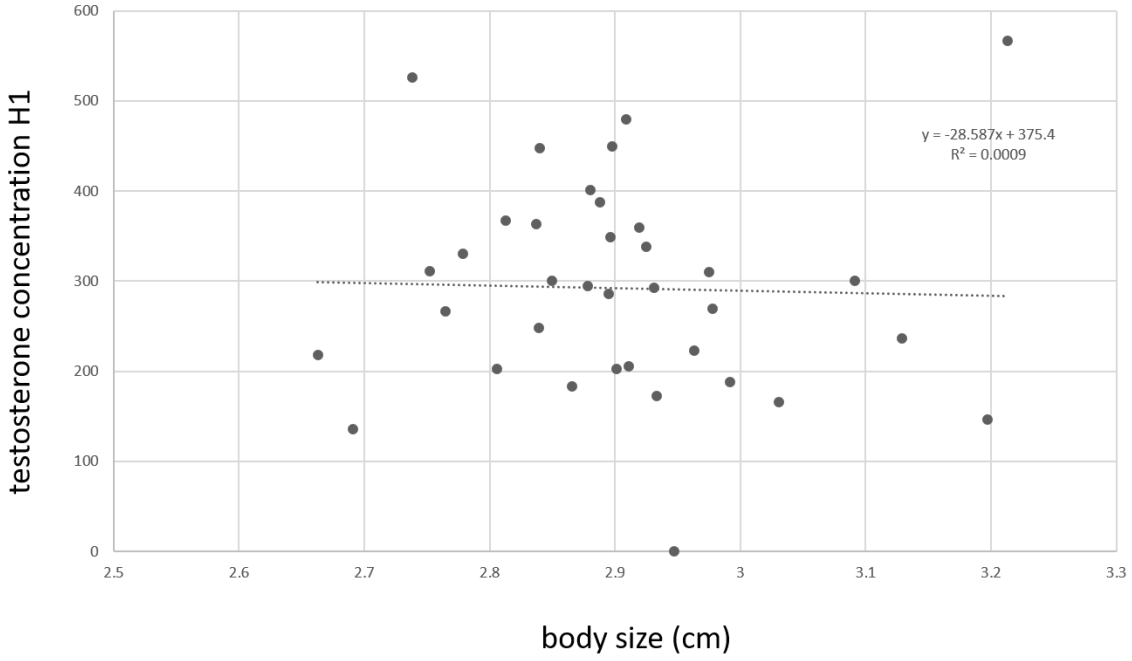


Figure 7.1.: The factor body size did not show any effect on testosterone concentrations in baseline measurements (H0).



Figures 7.2.: Body size did not show any influence on testosterone concentrations after behavioral tests (H1).

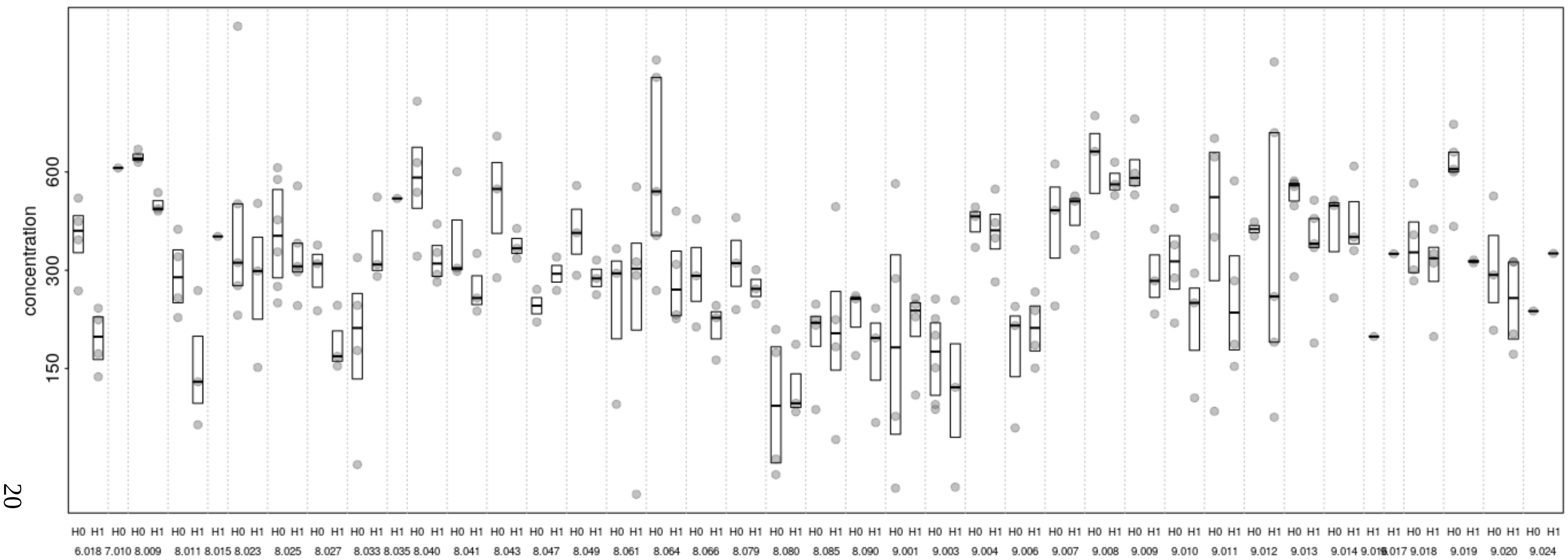


Figure 8: Inter- and intraindividual fluctuations of each individuals´ H0 and H1 measurements. from left to right: each individual is listed, 2 boxes per frog, one each per condition (H0/ H1); left box H0 = baseline measurements, right box H1 = measurements after behavioral experiments. Each box signifies the fluctuations of the respective measurements.

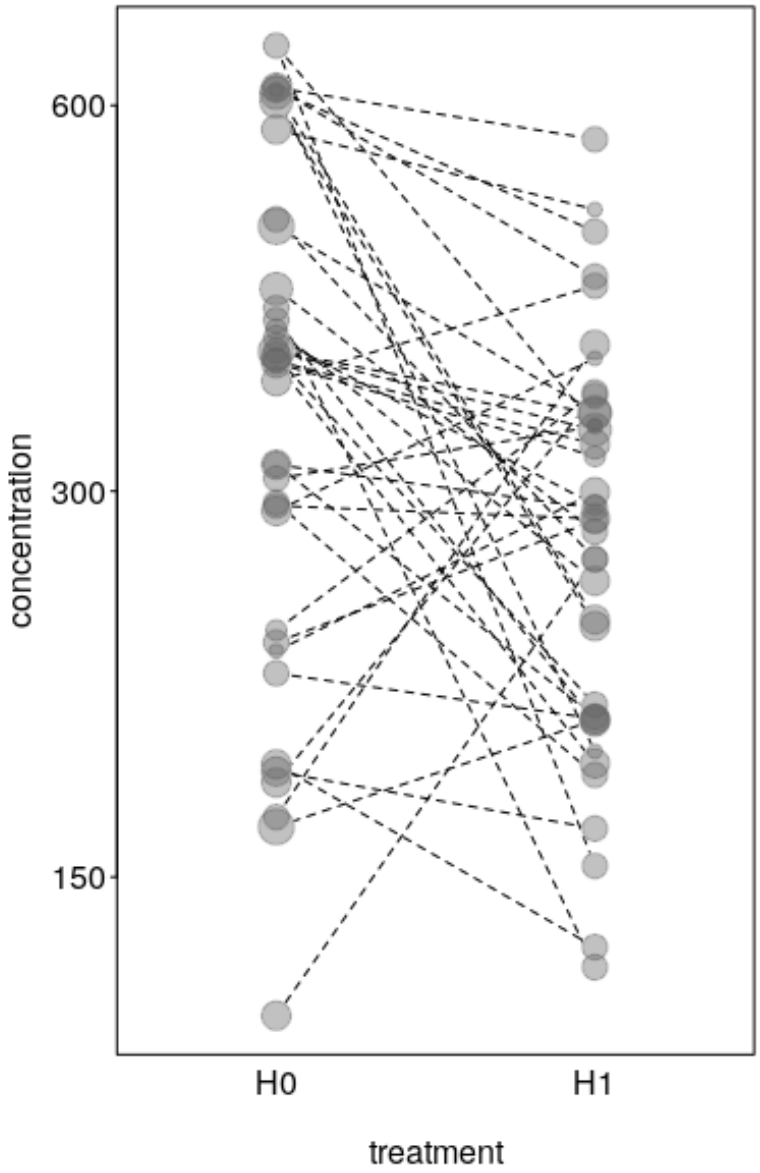


Figure 9: This figure shows a comparison between average testosterone levels of single individuals across the two treatments: H0 = baseline testosterone concentrations, H1 = concentration after behavioral experiments. Grey dots indicate average values of the respective measurements per condition. The bigger the dot, the more measurements were taken per frog. Each line between the dots connects the same individuals´ measurements of H0 and H1, to visualize the tendency of increase or decrease of the concentrations. There are two different dynamics: Individuals with low H0 concentrations showed the tendency of an increase in their H1 concentrations and Individuals with high H0 concentrations showed the tendency of a decrease in their H1 concentrations.

Discussion

The results of this study show that male *A. femoralis* have relatively consistent levels of testosterone (figure 8). The variation of testosterone concentrations in the water samples, within one (intra-individual) individual was lower than the variation of measurements compared within the population (inter-individual). Testosterone measurements were slightly but significantly lower after behavioral tests, compared to independent baseline concentrations (figure 4). We did not find any significant effect of diurnal rhythm, age or body size (figures 5,6,7) on testosterone. Interestingly, we found two different dynamics after behavioral tests: individuals with low baseline testosterone concentrations tended to have a higher testosterone response after the behavioral tests, while individuals with high baseline levels rather decreased their testosterone concentrations following the behavioral tests (figure 9).

Consistency of measurements

Figure 8 illustrates, that both, inter- and intra-individual testosterone concentrations stay relatively constant, with only a few exceptions. Those outliers could be due to situations which happened immediately before testosterone measurements. As we did not observe each frog for a long time prior to hormonal sampling, we cannot rule out that situations which happened immediately before collecting the data set (e.g. a recently ended courtship, a fight or other stressful situations), might have influenced the hormonal state of the frog. Nevertheless, even a low number of repeated measurements as in the present study seems to allow reliable estimations of individual hormonal profiles, since levels of testosterone were quite consistent over time, within and across individuals.

Influence of behavioral tests on testosterone levels

The behavioral experiments indeed had a significant effect on testosterone levels, as testosterone levels were on average lower after those tests compared to baseline levels. Social challenges are persistent in every aspect of an animals´ life cycles and demand immediate behavioral responses to find mating partners, assist in avoidance of predators and to search for food or other resources (Wingfield, Lynn, and Soma 2001). Those behaviors can be activated by hormones (Wingfield, Lynn, and Soma 2001) but likewise, behaviors can influence the hormonal state. That signifies that hormones and behavior are influenced in a bidirectional way (Soares et al. 2010). We found that testosterone concentrations were on average lower following

the behavioral experiments compared to the baseline testosterone levels (figure 4). This was surprising, as we expected the opposite effect (increased levels of testosterone after behavioral tests H1) according to the "Challenge Hypothesis" (Wingfield et al. 1990), which states the elevation of testosterone concentrations after a social challenge. An increase of testosterone has been observed many species following aggressive challenges (Wingfield and Farner 1978b; 1978a; Ros, Dieleman, and Groothuis 2002; Soto-Gamboa, Villalón, and Bozinovic 2005) However, in a study in *Hypsiboas faber* (de Assis et al. 2012) frogs were stimulated with a simulated territorial intrusion, similar to our test of aggressive behavior, and plasma testosterone was subsequently measured. Similar to the findings in our study, testosterone concentrations in *H. faber* also decreased after behavioral tests.

Figure 9 shows individual changes in average testosterone concentrations between both conditions (H0/H1) and two different dynamics in individual changes of testosterone levels seem to take place. On one hand, individuals with high baseline concentrations had the tendency to show a decrease in testosterone levels after behavioral experiments. On the other hand, individuals with low testosterone baseline levels showed the tendency to increase testosterone levels after behavioral experiments. This phenomenon could potentially be caused by individual differences in relation to personality. It may also be the case that differential personality types also show differential physiological (i.e. hormonal) responses to stress and/or social challenges (Baugh et al. 2012).

Investigations of the link between personality and hormones demand a carefully established experimental design. Since studies have shown that there is not just a unidirectional influence of hormones on behavior (Soares et al. 2010), it has become clear that levels of hormones can modulate behavior and vice versa. Therefore, the following question arises: when it is best to take hormonal samples? To minimize handling time and experimental effort, it would be ideal to measure hormonal levels directly before and/or after behavioral tests, however this approach could affect the hormonal and behavioural experiments, respectively. Whether hormonal measurements should be taken independently from behavioral tests, or also immediately thereafter, depends on the question of research. To gain information about long-term differences in hormonal profiles, measurements should be taken without any preceding behavioural experiments. However, when determining differences in physiological responses after behavioral challenges, it is important to carry out hormonal sampling completely independently from other experimental manipulations as well as directly after behavioural testing.

Circadian or Diurnal changes of testosterone levels

It is well known that many steroid concentrations undergo a circadian rhythm. In vertebrates and in humans, testosterone concentration rises up in early morning hours and drops down around midday/afternoon (Nelson 2011; Diver et al. 2003). Unfortunately, so far little has been investigated in frogs. Within this study we did not find any diurnal fluctuation of testosterone concentration (figure 5). It has to be mentioned that sampling times were not equal across conditions, as baseline measurements were conducted approximately 50% before noon (and 50% in the afternoon) and measurements after behavioral tests were exclusively taken in the afternoon, owing to the usual calling activity of the species. Since *A. femoralis* is a diurnal species, we commonly sampled the frogs from 8 a.m. to 12:30 a.m. and 2 a.m. to 7 p.m., according to their active hours. Thus, we cannot provide any information about changes in testosterone concentration during nighttime and midday. However, our data do not suggest any daily rhythm in testosterone concentrations of *Allobates femoralis* males. Several studies have documented the existence of diurnal cycles of circulating testosterone, for example in fish (Lorenzi et al. 2008), monkeys (Schlatt et al. 2008), or in young and middle-aged men (Diver et al. 2003) which show a peak of testosterone around 6 am. In turn, male green sea turtles did not show evidence for diurnal rhythms in testosterone (Licht, Wood, and Wood 1985).

Levels of steroid hormones generally vary between species/populations across geographic areas, elevations and different lengths of breeding season (Eikenaar et al. 2012). Vertebrates have shorter breeding seasons at higher latitude (temperate zone) in contrast to longer breeding seasons at lower latitudes (tropics) (Morrison & Hero 2003; Garamszegi et al. 2008). Shorter breeding seasons increase male-male competition, what may cause increased concentrations of testosterone and in further steps influences secondary sexual traits (Eikenaar et al. 2012). Increased energetic demands are an effect of higher reproductive intensity, which may cause shorter periods of breeding (Eikenaar et al. 2012). In amphibians, testosterone concentrations are often found to be positively related to latitude and negatively to the length of the breeding season (Eikenaar et al. 2012), which signifies that tropical animals usually exhibit lower testosterone levels with very low seasonal fluctuation during the reproductive season compared to temperate-zone, explosively breeding species. This could be a possible reason why we did not find any rhythmicity in testosterone concentrations over time in *Allobates femoralis* males in our study.

Influence of age and body size on testosterone levels

The factor "age" did not show significant effect on testosterone levels (figure 6). There is evidence for an age-related decrease of testosterone levels in various animal taxa (Schlatt et al. 2008; Morton et al. 1990), however those taxa typically show a greater lifespan than our studied species. It may be that age is not a relevant factor for variation in testosterone levels in short-lived species like *A. femoralis.*

Although in many animal species hormonal levels are related to animal size and thus animal growth (Bernstein et al. 2007), body size was not found to be correlated with testosterone levels in *A. femoralis*. This may result from the fact that the difference in body size between the smallest and the largest sampled individuals (approx. 5mm) was too small to be significant for testosterone differences.

Summary

This study shows that testosterone concentrations in *Allobates femoralis* remain constant within individuals and among the tested population (figure 8). Time of day, age and body size had no influence on testosterone concentrations. However, the performance of behavioral experiments had a strong effect on testosterone levels. Interestingly, the nature of the hormonal response appears to depend on individual baseline levels. Individuals with low baseline levels tended to increase testosterone concentrations and individuals with high baseline levels tended to reduce their testosterone concentration following the behavioral tests. These opposite hormonal responses to the behavioral tests, depending on the initial baseline levels, might be linked to differences in individual personality traits, a question which need to be investigated in future experiments.

Ethics

This study was approved by the scientific committee of the "Nouragues Ecological Research Station" and the ethics board of the University of Veterinary Medicine Vienna, as well as the University of Vienna. The hormonal such as the behavioral sampling was conducted in strict accordance with current French and EU law, according to the Study of Animal Behaviour (ASAB) guidelines.

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Appendix

ID	age	bodysize	H	date	month	ampm	order	time	conc
i16-m-018	1	2.972	H0	23.02.2019	february	pm	2	17:08	371.58
i16-m-018	1	2.972	H0	17.03.2019	march	am	4	9:24	498.8
i16-m-018	1	2.972	H0	26.03.2019	march	am	6	9:57	423
i16-m-018	1	2.972	H0	19.04.2019	april	am	8	10:35	259.42
i17-m-010	1	2.947	H0	15.02.2019	february	pm	1	16:56	617
i18-m-009	1	2.909	H0	08.04.2019	april	pm	1	17:00	703.6
i18-m-009	1	2.909	H0	13.04.2019	april	am	3	9:15	642.6
i18-m-009	1	2.909	H0	18.04.2019	april	pm	5	16:12	659.2
i18-m-011	1	3.031	H0	15.02.2019	february	pm	1	16:43	214.96
i18-m-011	1	3.031	H0	25.02.2019	february	pm	3	15:41	247.1
i18-m-011	1	3.031	H0	07.03.2019	march	pm	5	16:32	400.48
i18-m-011	1	3.031	H0	20.03.2019	march	am	7	11:17	330
i18-m-023	1	2.975	H0	21.02.2019	february	am	2	11:48	269.02
i18-m-023	1	2.975	H0	27.02.2019	february	am	4	11:20	218.58
i18-m-023	1	2.975	H0	09.03.2019	march	am	6	10:39	1675.6
i18-m-023	1	2.975	H0	12.04.2019	april	am	7	10:11	479.4
i18-m-023	1	2.975	H0	17.04.2019	april	pm	8	17:00	316.42
i18-m-025	1	2.896	H0	13.02.2019	february	pm	1	15:22	238.32
i18-m-025	1	2.896	H0	23.02.2019	february	am	3	10:12	267.56
i18-m-025	1	2.896	H0	16.03.2019	march	pm	5	16:40	618.2
i18-m-025	1	2.896	H0	14.04.2019	april	pm	7	16:01	569.2
i18-m-025	1	2.896	H0	18.04.2019	april	pm	9	16:29	341.58
i18-m-025	1	2.896	H0	21.04.2019	april	pm	11	15:53	428.2
i18-m-027	1	2.866	H0	07.04.2019	april	am	1	10:13	358.36
i18-m-027	1	2.866	H0	13.04.2019	april	am	3	9:37	314.26
i18-m-027	1	2.866	H0	18.04.2019	april	am	5	10:16	225.46
i18-m-033	1	2.813	H0	26.03.2019	march	am	2	9:37	234.08
i18-m-033	1	2.813	H0	31.03.2019	march	pm	4	16:05	328.06
i18-m-033	1	2.813	H0	09.04.2019	april	am	6	10:07	170.38
i18-m-033	1	2.813	H0	16.04.2019	april	am	7	10:32	76.24
i18-m-040	1	2.779	H0	06.03.2019	march	pm	2	15:23	640.4
i18-m-040	1	2.779	H0	18.03.2019	march	pm	4	17:19	519.8
i18-m-040	1	2.779	H0	26.03.2019	march	am	6	8:44	331.14
i18-m-040	1	2.779	H0	22.04.2019	april	pm	8	15:01	988.4
i18-m-041	1	2.978	H0	09.04.2019	april	pm	2	17:41	297.82
i18-m-041	1	2.978	H0	17.04.2019	april	am	4	9:01	601.2
i18-m-041	1	2.978	H0	22.04.2019	april	pm	6	14:55	304.42
i18-m-043	1	2.919	H0	09.04.2019	april	am	2	11:45	532.2
i18-m-043	1	2.919	H0	15.04.2019	april	am	4	9:52	772.2
i18-m-043	1	2.919	H0	21.04.2019	april	am	6	9:55	284.44
i18-m-047	1	2.878	H0	12.02.2019	february	pm	1	16:17	262
i18-m-047	1	2.878	H0	23.02.2019	february	am	3	10:38	208.38
i18-m-049	1	2.895	H0	21.03.2019	march	am	1	11:07	289.4
i18-m-049	1	2.895	H0	28.03.2019	march	pm	3	14:47	390.5
i18-m-049	1	2.895	H0	11.04.2019	april	pm	5	16:55	545
i18-m-061	1	2.963	H0	19.03.2019	march	am	1	11:32	293.5
i18-m-061	1	2.963	H0	26.03.2019	march	am	4	9:53	349.02
i18-m-061	1	2.963	H0	04.04.2019	april	pm	6	15:54	116.6
i18-m-064	1	3.091	H0	12.02.2019	february	pm	1	15:28	383.28
i18-m-064	1	3.091	H0	03.03.2019	march	pm	3	15:53	1168.8
i18-m-064	1	3.091	H0	12.03.2019	march	am	5	9:48	1321.4
i18-m-064	1	3.091	H0	07.04.2019	april	am	7	11:00	259.92
i18-m-064	1	3.091	H0	18.04.2019	april	pm	9	16:01	523
i18-m-066	1	2.806	H0	24.03.2019	march	pm	2	15:54	430
i18-m-066	1	2.806	H0	07.04.2019	april	am	4	10:30	288.5
i18-m-066	1	2.806	H0	13.04.2019	april	am	6	9:53	201.22
i18-m-077	1	NA	H0	21.04.2019	april	pm	2	16:17	64.74
i18-m-077	1	NA	H0	22.04.2019	april	am	3	10:27	98.82
i18-m-079	1	2.765	H0	13.03.2019	march	am	1	10:07	227.34
i18-m-079	1	2.765	H0	26.03.2019	march	pm	3	16:10	315.46
i18-m-079	1	2.765	H0	11.04.2019	april	pm	5	17:34	435.2
i18-m-080	1	2.691	H0	09.03.2019	march	pm	2	14:18	197.58
i18-m-080	1	2.691	H0	17.03.2019	march	am	4	9:45	167.86
i18-m-080	1	2.691	H0	23.03.2019	march	am	6	10:50	79.3
i18-m-080	1	2.691	H0	08.04.2019	april	am	7	10:25	71
i18-m-085	1	3.129	H0	10.02.2019	february	pm	2	15:52	210.74
i18-m-085	1	3.129	H0	23.03.2019	march	pm	4	16:33	203.58
i18-m-085	1	3.129	H0	30.03.2019	march	am	6	10:03	112.36
i18-m-085	1	3.129	H0	09.04.2019	april	am	7	10:40	236.12
i18-m-090	1	2.933	H0	15.02.2019	february	pm	1	17:06	164.56
i18-m-090	1	2.933	H0	22.03.2019	march	am	4	10:37	245.18
i18-m-090	1	2.933	H0	29.03.2019	march	am	6	9:50	250.68

i19-m-001	0	2.911	H0	13.02.2019	february	pm	1	14:17	552
i19-m-001	0	2.911	H0	13.03.2019	march	am	3	11:24	107.14
i19-m-001	0	2.911	H0	18.03.2019	march	am	5	10:59	64.52
i19-m-001	0	2.911	H0	07.04.2019	april	am	7	10:44	283.04
i19-m-003	0	3.197	H0	27.02.2019	february	am	2	12:07	116.44
i19-m-003	0	3.197	H0	12.03.2019	march	am	4	11:20	150.8
i19-m-003	0	3.197	H0	21.03.2019	march	pm	6	15:58	245
i19-m-003	0	3.197	H0	09.04.2019	april	pm	7	17:34	213.26
i19-m-003	0	3.197	H0	11.04.2019	april	pm	8	16:09	189.54
i19-m-003	0	3.197	H0	18.04.2019	april	pm	9	16:40	112.46
i19-m-004	0	2.880	H0	07.03.2019	march	pm	2	16:08	438.6
i19-m-004	0	2.880	H0	25.03.2019	march	am	4	9:30	352.4
i19-m-004	0	2.880	H0	11.04.2019	april	am	6	10:00	468.6
i19-m-006	0	2.901	H0	19.02.2019	february	pm	2	15:34	232.38
i19-m-006	0	2.901	H0	20.03.2019	march	pm	4	17:05	203.16
i19-m-006	0	2.901	H0	27.03.2019	march	am	6	9:04	98.72
i19-m-007	0	2.840	H0	08.03.2019	march	pm	2	17:15	233.12
i19-m-007	0	2.840	H0	29.03.2019	march	am	4	9:32	634.8
i19-m-007	0	2.840	H0	04.04.2019	april	pm	6	16:16	458
i19-m-008	0	3.213	H0	19.03.2019	march	pm	1	16:15	693.2
i19-m-008	0	3.213	H0	10.04.2019	april	pm	3	17:58	891.6
i19-m-008	0	3.213	H0	15.04.2019	april	am	5	11:13	383.96
i19-m-009	0	2.850	H0	11.04.2019	april	am	2	9:25	510
i19-m-009	0	2.850	H0	15.04.2019	april	am	4	9:08	872
i19-m-009	0	2.850	H0	19.04.2019	april	pm	5	13:47	594.6
i19-m-009	0	2.850	H0	21.04.2019	april	pm	7	14:46	556.4
i19-m-010	0	2.663	H0	13.02.2019	february	pm	1	14:42	464.6
i19-m-010	0	2.663	H0	07.03.2019	march	am	3	10:25	284.2
i19-m-010	0	2.663	H0	12.03.2019	march	am	5	10:29	206.78
i19-m-010	0	2.663	H0	07.04.2019	april	pm	7	16:18	358.9
i19-m-011	0	2.931	H0	16.03.2019	march	pm	2	16:45	378.9
i19-m-011	0	2.931	H0	22.03.2019	march	am	4	10:53	110.96
i19-m-011	0	2.931	H0	26.03.2019	march	am	6	9:05	666.2
i19-m-011	0	2.931	H0	29.03.2019	march	am	7	10:02	760.2
i19-m-012	0	2.738	H0	22.02.2019	february	pm	2	14:08	381.58
i19-m-012	0	2.738	H0	22.03.2019	march	pm	4	16:07	421.8
i19-m-013	0	2.837	H0	23.02.2019	february	pm	2	16:05	286.56
i19-m-013	0	2.837	H0	12.03.2019	march	am	4	10:22	472.6
i19-m-013	0	2.837	H0	31.03.2019	march	pm	6	16:27	554.2
i19-m-013	0	2.837	H0	09.04.2019	april	am	7	11:50	563.4
i19-m-013	0	2.837	H0	12.04.2019	april	am	8	9:59	541.2
i19-m-013	0	2.837	H0	16.04.2019	april	am	9	9:50	552.6
i19-m-014	0	2.898	H0	06.03.2019	march	pm	2	17:12	247
i19-m-014	0	2.898	H0	26.03.2019	march	am	4	10:55	492.2
i19-m-014	0	2.898	H0	04.04.2019	april	pm	6	16:00	473.4
i19-m-018	0	2.752	H0	19.03.2019	march	pm	2	17:05	300.8
i19-m-018	0	2.752	H0	22.03.2019	march	am	3	10:36	385.16
i19-m-018	0	2.752	H0	27.03.2019	march	am	5	8:43	278.52
i19-m-018	0	2.752	H0	16.04.2019	april	am	7	10:00	553.6
i19-m-019	0	2.888	H0	16.03.2019	march	am	1	10:39	408.74
i19-m-019	0	2.888	H0	27.03.2019	march	pm	3	15:40	839.8
i19-m-019	0	2.888	H0	05.04.2019	april	pm	5	16:30	690
i19-m-019	0	2.888	H0	16.04.2019	april	pm	6	16:04	600
i19-m-019	0	2.888	H0	19.04.2019	april	am	7	11:14	612
i19-m-020	0	2.839	H0	18.03.2019	march	pm	2	16:13	506.2
i19-m-020	0	2.839	H0	30.03.2019	march	am	4	9:35	290.38
i19-m-020	0	2.839	H0	15.04.2019	april	am	6	10:55	196.5
i19-m-024	0	2.925	H0	06.04.2019	april	pm	2	16:00	225.04
i16-m-018	1	2.972	H1	11.02.2019	february	pm	1	17:00	141.52
i16-m-018	1	2.972	H1	10.03.2019	march	pm	3	15:39	229.58
i16-m-018	1	2.972	H1	24.03.2019	march	pm	5	17:44	166.56
i16-m-018	1	2.972	H1	15.04.2019	april	pm	7	16:34	211.6
i18-m-009	1	2.909	H1	10.04.2019	april	pm	2	18:08	519
i18-m-009	1	2.909	H1	15.04.2019	april	pm	4	17:07	455.4
i18-m-009	1	2.909	H1	20.04.2019	april	pm	6	15:10	463
i18-m-011	1	3.031	H1	20.02.2019	february	pm	2	18:00	100.88
i18-m-011	1	3.031	H1	03.03.2019	march	pm	4	14:08	259.86
i18-m-011	1	3.031	H1	15.03.2019	march	am	6	11:44	136.66
i18-m-015	1	3.063	H1	19.02.2019	february	pm	1	17:04	380.82
i18-m-023	1	2.975	H1	12.02.2019	february	pm	1	16:04	480.8
i18-m-023	1	2.975	H1	25.02.2019	february	pm	3	18:08	151.26
i18-m-023	1	2.975	H1	07.03.2019	march	pm	5	16:45	298.32
i18-m-025	1	2.896	H1	21.02.2019	february	pm	2	16:46	543.6
i18-m-025	1	2.896	H1	03.03.2019	march	pm	4	14:50	363.06
i18-m-025	1	2.896	H1	19.03.2019	march	pm	6	15:47	308.32
i18-m-025	1	2.896	H1	16.04.2019	april	pm	8	16:09	296.28
i18-m-025	1	2.896	H1	20.04.2019	april	pm	10	15:37	233.82

i18-m-027	1	2.866	H1	10.04.2019	april	pm	2	17:00	234.2
i18-m-027	1	2.866	H1	15.04.2019	april	pm	4	16:07	163.58
i18-m-027	1	2.866	H1	20.04.2019	april	pm	6	16:23	152.62
i18-m-033	1	2.813	H1	24.03.2019	march	pm	1	16:59	502.8
i18-m-033	1	2.813	H1	29.03.2019	march	pm	3	17:45	312.52
i18-m-033	1	2.813	H1	07.04.2019	april	pm	5	15:30	287.44
i18-m-035	1	2.982	H1	10.02.2019	february	pm	1	17:00	497.6
i18-m-040	1	2.779	H1	03.03.2019	march	pm	1	17:14	291.34
i18-m-040	1	2.779	H1	12.03.2019	march	pm	3	16:42	415.6
i18-m-040	1	2.779	H1	24.03.2019	march	pm	5	16:47	339.8
i18-m-040	1	2.779	H1	16.04.2019	april	pm	7	17:04	275.94
i18-m-041	1	2.978	H1	07.04.2019	april	pm	1	17:15	224.88
i18-m-041	1	2.978	H1	15.04.2019	april	pm	3	17:30	246.68
i18-m-041	1	2.978	H1	20.04.2019	april	pm	5	15:59	337.66
i18-m-043	1	2.919	H1	07.04.2019	april	pm	1	15:24	326
i18-m-043	1	2.919	H1	14.04.2019	april	pm	3	16:15	402.6
i18-m-043	1	2.919	H1	19.04.2019	april	pm	5	16:33	350.34
i18-m-047	1	2.878	H1	20.02.2019	february	pm	2	17:35	260.26
i18-m-047	1	2.878	H1	03.03.2019	march	pm	4	15:33	329.14
i18-m-049	1	2.895	H1	25.03.2019	march	pm	2	17:40	252.5
i18-m-049	1	2.895	H1	01.04.2019	april	pm	4	16:55	322.2
i18-m-049	1	2.895	H1	13.04.2019	april	pm	6	17:08	283.26
i18-m-061	1	2.963	H1	17.03.2019	march	pm	2	16:46	539.8
i18-m-061	1	2.963	H1	24.03.2019	march	pm	3	16:52	318.22
i18-m-061	1	2.963	H1	01.04.2019	april	pm	5	18:17	289.34
i18-m-061	1	2.963	H1	21.04.2019	april	pm	7	16:15	61.82
i18-m-064	1	3.091	H1	22.02.2019	february	pm	2	16:18	454.8
i18-m-064	1	3.091	H1	06.03.2019	march	pm	4	18:36	219.1
i18-m-064	1	3.091	H1	19.03.2019	march	pm	6	15:42	213.2
i18-m-064	1	3.091	H1	16.04.2019	april	pm	8	17:21	312.8
i18-m-066	1	2.806	H1	12.03.2019	march	pm	1	17:20	214.56
i18-m-066	1	2.806	H1	27.03.2019	march	pm	3	15:45	233.88
i18-m-066	1	2.806	H1	10.04.2019	april	pm	5	16:29	159.28
i18-m-079	1	2.765	H1	22.03.2019	march	pm	2	17:11	263.12
i18-m-079	1	2.765	H1	07.04.2019	april	pm	4	16:10	300.86
i18-m-079	1	2.765	H1	14.04.2019	april	pm	6	17:30	236.22
i18-m-080	1	2.691	H1	07.03.2019	march	pm	1	16:16	110.62
i18-m-080	1	2.691	H1	15.03.2019	march	pm	3	16:46	177.96
i18-m-080	1	2.691	H1	21.03.2019	march	pm	5	17:15	117.48
i18-m-085	1	3.129	H1	07.03.2019	march	pm	1	18:04	174.74
i18-m-085	1	3.129	H1	20.03.2019	march	pm	3	16:46	211.8
i18-m-085	1	3.129	H1	26.03.2019	march	pm	5	15:53	469.4
i18-m-085	1	3.129	H1	22.04.2019	april	pm	8	16:07	90.94
i18-m-090	1	2.933	H1	15.03.2019	march	pm	2	17:00	186.12
i18-m-090	1	2.933	H1	19.03.2019	march	pm	3	16:02	229.58
i18-m-090	1	2.933	H1	25.03.2019	march	pm	5	18:01	102.54
i19-m-001	0	2.911	H1	09.03.2019	march	pm	2	17:21	246.72
i19-m-001	0	2.911	H1	15.03.2019	march	pm	4	17:33	215.86
i19-m-001	0	2.911	H1	20.03.2019	march	pm	6	16:20	236
i19-m-001	0	2.911	H1	16.04.2019	april	pm	8	17:42	124.42
i19-m-003	0	3.197	H1	25.02.2019	february	pm	1	17:42	131.42
i19-m-003	0	3.197	H1	09.03.2019	march	pm	3	16:32	65
i19-m-003	0	3.197	H1	19.03.2019	march	pm	5	15:30	242.92
i19-m-004	0	2.880	H1	19.02.2019	february	pm	1	14:55	376.64
i19-m-004	0	2.880	H1	21.03.2019	march	pm	3	17:44	531.4
i19-m-004	0	2.880	H1	09.04.2019	april	pm	5	17:27	419.6
i19-m-004	0	2.880	H1	19.04.2019	april	pm	7	15:46	276.32
i19-m-006	0	2.901	H1	13.02.2019	february	pm	1	15:47	176.86
i19-m-006	0	2.901	H1	06.03.2019	march	pm	3	18:10	150.44
i19-m-006	0	2.901	H1	25.03.2019	march	pm	5	16:49	257.5
i19-m-006	0	2.901	H1	18.04.2019	april	am	7	10:45	225.62
i19-m-007	0	2.840	H1	06.03.2019	march	pm	1	16:42	347.48
i19-m-007	0	2.840	H1	27.03.2019	march	pm	3	15:18	506.6
i19-m-007	0	2.840	H1	01.04.2019	april	pm	5	16:32	487.8
i19-m-008	0	3.213	H1	24.03.2019	march	pm	2	16:16	509
i19-m-008	0	3.213	H1	12.04.2019	april	pm	4	16:44	549.8
i19-m-008	0	3.213	H1	20.04.2019	april	pm	6	17:29	642.8
i19-m-009	0	2.850	H1	07.04.2019	april	pm	1	15:30	220.44
i19-m-009	0	2.850	H1	13.04.2019	april	pm	3	16:24	278.38
i19-m-009	0	2.850	H1	20.04.2019	april	pm	6	15:12	401.5

i19-m-010	0	2.663	H1	25.02.2019	february	pm	2	17:49	122.02
i19-m-010	0	2.663	H1	09.03.2019	march	pm	4	14:58	238.4
i19-m-010	0	2.663	H1	19.03.2019	march	pm	6	16:32	293.62
i19-m-011	0	2.931	H1	13.03.2019	march	pm	1	18:20	152.24
i19-m-011	0	2.931	H1	20.03.2019	march	pm	3	16:38	177.94
i19-m-011	0	2.931	H1	24.03.2019	march	pm	5	17:56	278.54
i19-m-011	0	2.931	H1	08.04.2019	april	am	8	9:56	563.4
i19-m-012	0	2.738	H1	19.02.2019	february	pm	1	16:31	180.68
i19-m-012	0	2.738	H1	15.03.2019	march	pm	3	17:28	791.4
i19-m-012	0	2.738	H1	26.03.2019	march	pm	5	16:10	1303
i19-m-012	0	2.738	H1	11.04.2019	april	pm	6	17:55	106.44
i19-m-012	0	2.738	H1	13.04.2019	april	pm	7	17:30	249.42
i19-m-013	0	2.837	H1	12.02.2019	february	pm	1	17:28	352.12
i19-m-013	0	2.837	H1	07.03.2019	march	pm	3	17:09	432.4
i19-m-013	0	2.837	H1	26.03.2019	march	pm	5	17:40	179.58
i19-m-013	0	2.837	H1	17.04.2019	april	pm	10	16:40	361.88
i19-m-013	0	2.837	H1	21.04.2019	april	pm	11	15:24	491.6
i19-m-014	0	2.898	H1	13.02.2019	february	pm	1	15:26	379.24
i19-m-014	0	2.898	H1	24.03.2019	march	pm	3	15:33	624.8
i19-m-014	0	2.898	H1	01.04.2019	april	pm	5	17:52	344.7
i19-m-016	0	2.992	H1	13.02.2019	february	pm	1	17:00	187.98
i19-m-017	0	2.687	H1	19.02.2019	february	pm	1	17:14	336.92
i19-m-018	0	2.752	H1	13.03.2019	march	pm	1	17:53	401.02
i19-m-018	0	2.752	H1	24.03.2019	march	pm	4	17:24	337.88
i19-m-018	0	2.752	H1	31.03.2019	march	pm	6	17:28	187.96
i19-m-018	0	2.752	H1	17.04.2019	april	pm	8	16:15	315.64
i19-m-019	0	2.888	H1	24.03.2019	march	pm	2	17:15	322.66
i19-m-019	0	2.888	H1	31.03.2019	march	pm	4	17:12	316.08
i19-m-020	0	2.839	H1	09.03.2019	march	pm	1	15:29	318.7
i19-m-020	0	2.839	H1	20.03.2019	march	pm	3	15:30	166.02
i19-m-020	0	2.839	H1	21.04.2019	april	pm	5	17:46	191.38
i19-m-020	0	2.839	H1	22.04.2019	april	pm	7	17:30	317.92
i19-m-024	0	2.925	H1	31.03.2019	march	pm	1	17:42	337.82