Master's Thesis

Communication between the tadpoles of the dyeing poison frog *Dendrobates tinctorius* (Anura, Dendrobatidae)

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Communication between animals is fundamental in the context of mate choice, predator avoidance and territory defense, among others. The oldest and most widespread mode of animal communication is chemical communication, which is particularly useful in aquatic environments. In animals that are fully aquatic for only part of their lives, like amphibians, chemical communication at the larval stage is interesting because it has not evolved for an immediate sexual advantage, making behavioural assays easier to interpret. *Dendrobates tinctorius* is a neotropical poison frog whose males transport tadpoles to small water pools where they develop until metamorphosis. This study aims to determine with behavioral laboratory experiment which communication mode(s) are used by *D. tinctorius* tadpoles to detect another individual. I hypothesized that tadpoles use a combination of visual and chemical cues for that purpose, as shown in other poison frog species. This detection should manifest as an increased activity of tadpoles. Surprisingly, I found that tadpoles are the most active when exposed only to visual cues, indicating that tadpoles might be more motion-orientated than previously thought. Tadpoles exposed exclusively to visual cues also differed in their space use when compared to the control group (exposed to no cues): they spend more time closer to the individual they are seeing, which indicates that they are using their vision to gather more information about their surroundings and possible threats.

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Hakusanat: aisti, hajuaisti, käyttäytymisekologia, näköaisti

Eläimet tarvitsevat kommunikointia eri tarkoituksiin, kuten parin muodostukseen, saalistajan havaitsemiseen ja reviirin hallintaan. Kemiallinen kommunikointi on kommunikoinnin muodoista vanhin ja yleisin. Sammakkoeläimet, ja erityisesti niiden akvaattiset nuijapäät, soveltuvat hyvin kemiallisen kommunikoinnin tutkimukseen. Nuijapäiden käytös ei tähtää välittömään seksuaaliseen hyötyyn, joten käyttäytymistä on helpompi tulkita. *Dendrobates tinctorius* on myrkkysammakko, jonka nuijapäät kehittyvät sademetsässä pienissä vesialtaissa metamorfoosiin asti. Tämä tutkimus pyrkii selvittämään laboratoriossa suoritetun käyttäytymiskokeen avulla aistiärsykkeen, jonka avulla *D. tinctorius* -lajin nuijapää havaitsee toisen yksilön läsnäolon (näkö, haju vai näiden yhdistelmä). Tutkimuksen hypoteesi oli, että nuijapäät käyttävät näkö- ja hajuaistin yhdistelmää lajitoverin tunnistamiseen, kuten toisella myrkkysammakkolajilla on tutkittu tapahtuvan. Reagointi lajitoveriin nähdään nuijapään aktiivisuuden lisääntymisenä. Tulosten perusteella nuijapäät olivat kuitenkin aktiivisimpia, kun ne ainoastaan näkivät toisen yksilön. Mahdollinen selitys on, että näkö on nuijapäille tärkeämpi aisti kuin aiemmin on ajateltu. Tulosten perusteella nuijapäät, jotka vain näkivät toisen yksilön, erosivat tilan käytöltään kontrollista viettäen enemmän aikaa altaan reunassa lähellä toista yksilöä. Tämä viittaa siihen, että nuijapäät hyödyntävät näköään ympäristönsä havainnointiin.

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1 INTRODUCTION

The study of animal behavior encompasses all the ways in which animals interact with their physical environment and other organisms (Davies et al. 2012). These interactions are vital for shaping mate choice, cooperation, and aggression between individuals, and ultimately represent different forms of inter- or intraspecific communication. Animal communication is defined by the action of one individual altering the pattern of behavior in another individual (Wilson 1970). Communication can be broadly classified in two categories: active and passive (Maynard Smith and Harper 2003). Signals are released to the environment intentionally to be detected by another animal, and are beneficial to the emitter and, on average, to the receiver (Maynard Smith and Harper 2003). Cues, on the other hand, are released to the environment unintentionally by the animal and transfer information, but are not selected for that function (Steiger et al. 2011). A receiver's reaction to an emitter's cue can be selectively neutral, negative or beneficial (Steiger et al. 2011). If the reaction is beneficial for the emitter, a cue might become a signal (Wyatt 2010).

Most signals used in animal communication belong to four fundamental sensory modalities, i.e., auditory (e.g. anuran calls and birdsong: Narins et al. 2006, Todt and Naguib 2000), visual (e.g. female stalk-eyed flies prefer large eye spans and colour polymorphism in Gouldian finches: Chapman et al. 2005, Pryke and Griffith 2006), chemical (e.g. honey bees using alarm pheromones and species recognition in sac winged bats: Li et al. 2014, Caspers et al. 2009) or tactile (e.g. grooming among langurs and object localization by cockroach antennae: Weber 1973, Okada and Toh 2006). The type(s) of signals that can be emitted by an animal depends on what is energetically possible to produce and is ultimately defined by the biology of the species (Endler 1993). For example, if the species is nocturnal, like most terrestrial mammals are, visual displays are not a very effective communication modality and, thus, not favored by natural selection (Campbell et al. 2017). Thus, it is important to consider all the characteristics of a species' life strategy and habitat, when exploring its communication modes.

1.1 Chemical communication

Chemical communication is the oldest and most widespread mode of communication among organisms (Wilson 1970). The chemicals used in animal communication are broadly defined as semiochemicals (Law and Regnier 1971). Pheromones are used in intraspecific chemical communication, whereas allelochemicals are used in interspecific communications (Wyatt 2003). Further, allelochemicals can be divided into allomones, which benefit the emitter (Brown Jr. 1968), kairomones, which benefit receivers (Brown Jr. et al. 1970), and synomones which benefit both participants (Nordlund and Lewis 1976).

Chemical signals can be emitted and received across a variety of environments. In aquatic environments the role of chemical communication can be particularly important as visibility breaks down under turbid conditions and the use of acoustic communication is limited in shallow waters (1 m–2 m), due to sound waves being unable to propagate when the water depth is lower than the wavelength (Wisenden 2000, Hemilä and Reuter 2008, Ladich and Winkler 2017). In addition, aquatic environments are well suited for chemical compounds to dissolve and disperse (Wisenden 2000). For example, fish use chemical communication to avoid predators (e.g. Brown et al. 1995), to detect and avoid injured conspecifics (e.g. Brown and Smith 1997) and to choose mates (e.g. Giaquinto et al. 2010). Another group that inhabits aquatic environments, at least during the early stages of their life, are anurans (frogs and toads). Anurans have a complex life cycle which involves, in most cases, the metamorphosis of aquatic larvae to semi- or fully terrestrial adults (Wells 2007). Because of this drastic change in their habitat across different life stages, it is likely that their most optimal communication mode changes throughout development. Although anurans are especially known for their vocal behavior in their adult stages (Narins et al. 2006), it has been shown that some species rely on other communication modes – including chemical communication (see review Starnberger et al. 2014).

Observations made by Rabb and Rabb (1963) proved that at least some adult anurans probably use chemical cues in their sexual communication. Researchers noticed changes in the behavior (agitation, calling) of unmated *Pipa pipa* males when frogs were exposed to water in which mating pairs had been earlier. However, the first sex pheromone from an anuran was only identified some thirty years later by Wabnitz et al. (1999); the identified pheromone was produced by males of the magnificent tree frog (*Litoria splendida*), and hence named splendipherin. Chemical information is also used for orientation in some anuran species, for example to find breeding pools or home ranges (e.g *Incilius valliceps*: Grubb 1970, *Anaxyrus fowleri*, *Pseudacris clarkii*, *Pseudacris streckeri*: Grubb 1973, *Oophaga pumilio*: Forester and Wisnieski 1991).

Chemical communication is not limited to anuran adult stages. Studies have shown that tadpoles from some bufonid and ranid species can, like some fish, detect alarm substances from injured conspecifics, which can cause behavioral changes or even earlier metamorphosis in tadpoles (e.g. Hews and Blaustein 1985, Kiesecker et al. 1999, Kiesecker et al. 2002). Pfennig et al. (1993) have also observed that cannibalistic tadpoles of the Spadefoot toad, *Scaphiopus bombifrons*, show less aggressive behavior towards kin. In this system, researchers established that kinship is determined by taste, where tadpoles will nip each others' bodies, suggesting that chemical cues might be used in kin recognition.

In the tropics, where anuran species diversity is high, many tadpoles develop in small pools of water, in which the risk of predation might be lower than in ponds or swamps (Wells 2007). Despite the small water volumes in which some tadpoles develop, chemical communication is still used also among frog tadpoles. Kam and Yang (2002) observed that the activity of tadpoles of a Taiwanese tree frog (*Chirixalus eiffingeri*) increased significantly when female frogs were present, and tadpoles even nipped females' skin. By doing this, tadpoles were begging a female to lay eggs for them to eat. When only visual cues were present, tadpoles did not show any response to the presence of a female frog. These alterations in *C. eiffingeri* tadpole behavior suggest that they use chemical and tactile cues to communicate with the female frog. The strawberry poison dart frog tadpoles (*Oophaga pumilio*), recognize females and beg for nutritive eggs by using a combination of chemical, visual and tactile cues

(Stynoski and Noble 2012). These studies demonstrate the role of chemical cues in parent-offspring interactions. Coupled with kin recognition work between tadpoles in closely related anurans, previous work establishes a strong foundation for the possible role of chemical communication between tadpoles in tropical poison frogs.

1.2 Poison frogs

Poison frogs are a group of anurans that live in the tropical region of South and Central America (Myers and Daly 1983). There are over 300 known species of poison frogs (Frost 2020), which belong to the superfamily Dendrobatoidea. The superfamily includes two families: Aromobatidae and Dendrobatidae (Grant et al. 2006). However, there is no such a term as "the poison frog" as a single functional type (Lötters et al. 2007).

Despite that, some common characteristics to all poison frogs can be defined. Poison frogs are small to medium size (2 cm–6 cm) (Lötters et al. 2007) and most of them are diurnal (Grant et al. 2006). Poison frog species can be classified based on their colour and toxicity: roughly one third of the known species are aposematic and produce defensive compounds (Summers and Clough 2001, Santos et al. 2003, Vences et al. 2003). The rest of the species are inconspicuously coloured and nontoxic (Santos et al. 2016). Poison frogs have well-developed social and reproductive behavior, which vary enormously across taxa, making them an interesting family to study (Myers and Daly 1983).

Poison frogs differ from most frogs with respect to their egg-laying behavior – they lay their eggs terrestrially, whereas most of the others lay them in water (Myers and Daly 1983, Summers and McKeon 2004). Because eggs are laid on the ground, they need to be transported by parents to bodies of water after hatching. Depending on the species, parental care can be performed by mothers, fathers or both parents (Summers and McKeon 2004); overall, across the poison frog superfamily, care is subject to an impressive range of interspecies variation.

Dendrobates tinctorius is a poison frog species that belongs to the family Dendrobatidae (Noonan and Gaucher 2006). In the wild this species is found around canopy gaps of primary forests in the eastern part of Guiana Shield, and can be found in ranges from sea level to 600 meters in elevation (Noonan and Gaucher 2006, Born et al. 2010). The species is poisonous in the wild, obtaining its toxins from alkaloids sequestered from their diet (Santos et al. 2003). *D. tinctorius* differs from most other frogs by not producing advertisement calls (Rojas and Pašukonis 2019), which suggests that other communication modes might have an important role in this species' social behavior.

D. tinctorius males perform parental care by transporting recently hatched tadpoles, one or two at the time, to suitable bodies of water, such as holes in standing or fallen trees (Rojas 2014). Tadpoles are left in the pools unattended until they metamorphose (Rojas 2014). Across the poison frog family many tadpoles are cannibalistic (Caldwell and De Araújo 1998, Summers 1999). As a result of this behavior, parents usually avoid already occupied phytotelmata (Weygoldt 1980, Caldwell and De Araújo 1998, Summers 1999), though exceptions to this trend exist (Rojas 2014). Schulte and Lötters (2013) found that adult *Ranitomeya variabilis* use chemical cues produced by conspecific tadpoles to avoid already occupied pools when depositing their own tadpoles. However, Rojas (2014) found that *D. tinctorius* males do not seem to avoid already occupied pools. Instead, they even tend to favor pools that are occupied by a large conspecific. Overall, the presence of another, larger tadpole may provide information to the father about pool quality and stability, which may be a more valuable pool cue than the potential risk of being cannibalized (Rojas 2014).

1.3 Communication between tadpoles

Tadpoles can serve as model organisms to study communication as, in this life stage, tadpole behavior is mainly restricted to activities that enhance development, growth, and survival to metamorphosis (Hoff et al. 1999); thus, it can be said that communication between individuals has not evolved for an immediate sexual advantage, making behavioural assays easier to interpret.

Tadpoles develop in aquatic environments (Wells 2007) and, due to the visual and acoustic challenges they change in these environments, chemical cues are critical for their communication (Wisenden 2000). Their eyes are know to be functional to some degree (e.g., Rot-Nikcevic et al. 2005, Gouchie et al. 2008), although it has been shown that tadpoles are probably near-sighted, meaning that the detection of anything from great distances or with much precision based on vision is unlikely (Mathis et al. 1988). Regarding the anatomy of the tadpole ear, it has been shown that it differs from the adult's and that, during metamorphosis, tadpole auditory sensitivity goes under a lot of changes including a brief "deaf" period (Boatright-Horowitz and Simmons 1997). However, tadpoles can detect sounds shortly after hatching until just before metamorphic climax, although with lower sharpness of tuning than froglets (Boatright-Horowitz and Simmons 1997).

Chemical cues, in turn, can stimulate changes in behavioural decisions when received by the tadpole (Petranka et al. 1987, Horat and Semlitsch 1994, Eklöv 2000). For example, when a tadpole detects chemical cues from a predator, it may react by minimizing its activity or stopping all movement in order to lessen the chance of being detected and eaten by the predator (Lawler 1989, Skelly 1994). The tadpoles of many species are also able to recognise their kin using chemical information (e.g. *Rana cascadae*: Blaustein and O'Hara 1982, *Anaxyrus americanus*: Waldman 1985, *Scaphiopus bombifrons*: Pfennig et al. 1993). For example, Dulcis et al. (2017) showed that long exposure to kin or non-kin odorants changes the number of dopamine -or gamma aminobutyric acid -expressing neurons with corresponding changes in attraction/aversion behavior in *Xenopus laevis* larvae. In addition, a study performed by Crossland and Shine (2011) revealed that cane toad tadpoles (*Bufo marinus*) use chemical signals to locate conspecific late-stage eggs which they then consume. These examples highlight the importance of olfaction and chemical information across diverse tadpole species.

Tadpoles of *Dendrobates tinctorius* develop in phytotelmata (small water holdings in the vegetation), where they are deposited by their fathers (Rojas 2014). *D. tinctorius* tadpoles feed on detritus, insect larvae, and the eggs and tadpoles of frogs (Rojas 2014). Tadpoles are cannibalistic and frequently interact (Rojas 2014, Rojas 2015). Because tadpoles are confined to their nursery after transport, and phytotelmata nurseries are generally low-nutrient, high-risk environments (Gray et al. 2009), cannibalism provides both nutrients for fast growth (Crump 1990) and a way to remove competition and possible predators (other cannibalistic tadpoles, Gray et al. 2009). The rates and latency of cannibalism in the wild are variable, suggesting that some kind of mechanism is driving interactions between tadpoles. From a proximate perspective, it has been found that the neural activity between tadpoles who have engaged in aggressive encounters differs from those who have not; on a finer scale, brain activity even differs between winners and losers of antagonistic interactions (Fischer et al. 2020). Despite recent interest in the proximate and ultimate approaches of understanding the basis of tadpole aggression, it is still unsure what modalities tadpoles use to detect and assess one another. To explore this fundamental level of communication between tadpoles, this study aims to understand how tadpoles assess each other when living in the same habitat.

Individuals gather information about their surroundings through their senses, and such information may cause alterations in the individual's behaviour. Understanding the sensory modality (or modalities) which trigger activity in tadpoles, when only conspecifics are present, will both help me understand how tadpoles recognize the presence of each other and the mechanisms by which this takes place. Both abilities are essential for a tadpole: they need to assess and react to the risk posed by a conspecific in order to survive until metamorphosis. While tadpole-parent communication (Kam and Yang 2002, Stynoski and Noble 2012, Schulte and Lötters 2013) and tadpole-predator interactions (Lawler 1989, Skelly 1994, Griffiths et al. 1998, Eklöv 2000, Bridges 2002, Crane and Ferrari 2017) have been studied, so far tadpole-tadpole communication has not received as much attention.

I studied *D. tinctorius* tadpoles in laboratory conditions to gain a better understanding of their social behavior. This study addresses punctual questions about intraspecific communication, especially at the larval stage, thus providing insight into the importance of different sensory modalities for poison frog tadpoles.

1.4 Research questions and hypothesis

The goal of this study was to determine which communication mode(s) are used by *D. tinctorius* tadpoles in detecting another individual. This was done by exposing tadpoles to visual, chemical or bimodal (visual + chemical) cues, and assessing tadpole activity. Because chemical cues reveal information about the presence or absence of threats and visual cues deliver immediate information of the threat position, I hypothesized that the combination of visual and chemical cues would trigger the most activity in tadpoles. In this study increased tadpole activity, meaning more time spent swimming than resting, was used to indicate detection of another individual. When meeting another individual, tadpoles should increase their activity to enhance foraging, since they are competing for the same resources (Werner and Anholt 1993). Also, as mentioned, this species is cannibalistic, so increased activity can be also expected as behavior prior to aggression. Thus, according to my hypothesis, tadpoles should spend more time swimming than resting when exposed to visual and chemical cues from another tadpole.

2 MATERIALS AND METHODS

2.1 Husbandry

Tadpoles of *D. tinctorius* raised in laboratory conditions in the facilities of the University of Jyväskylä were used in this study. The species' clutch size is small, varying between 2–5 eggs in the natural habitat (Rojas and Pašukonis 2019). In captivity clutches appear to be larger, from 4 to 11 eggs (personal observation). In captivity males transport the hatched tadpoles to small plastic containers, from which tadpoles are moved away by hand after the whole clutch has been transported. After that tadpoles are raised singly in small plastic containers (10 × 6.5 × 5 cm) filled with spring water. If a frog pair lays more than one clutch, the more recently laid one is removed and cared for by hand. In the laboratory tadpoles are fed *ad libitum* with JBL Novo Vert flakes (originally designed for plant-eating aquarium fish) three times a week.

2.2 Assays with tadpoles

Tadpole activity can be influenced by body size, so their weight was measured (digital pocket scale, range: 0.01 g–200 g) prior to the experiment. During the experiment tadpoles were kept in plastic water containers with a size of 16 × 11 × 10 cm (l × w × h). Water in the containers was spring water kept at room temperature. Because it is known that kinship may affect tadpole activity (Fouilloux et al. 2020, in press), the experiment was repeated 10 times for siblings and non-siblings (N = 20 total trials). Tadpoles were used only in one trial to remove possible carry-on effects on behavior. To perform all replicates of the treatments for both kin levels, 80 tadpoles were used in the study (4 tadpoles per trial). Tadpoles were chosen randomly, but sometimes were limited to the breeding of adult pairs. Sibling and non-sibling treatments were conducted in random order. In addition, within one trial, tadpoles were randomly assigned to different experimental treatments.

During each trial, each of the four tadpoles was exposed to either visual, chemical, bimodal (visual + chemical) or no cues for 45 minutes. Tadpole activity was videotaped (Canon Legria HF R46, 25 fps) and all occurrences of tadpole movement and the time that tadpoles spent resting or swimming (moving with flexible tail movements) were determined using the software BORIS (v. 7.9.19, Friard and Gamba 2016).

The set-up of a experimental trial consisted of a large plastic tank (42 × 15 × 28 cm) that was filled with 2.7 l tap water in which four smaller plastic containers were held (16 × 11 × 10 cm). These small containers formed a rectangle; in each container, two walls were covered with opaque plastic, so that every tadpole was exposed to different cues (Fig. 1). The final volume of all of the smaller plastic containers was 500 ml. Odor-dependent treatments had 400 ml of fresh spring water to which 100 ml of cue-containing experimental water was added immediately before the trial began. The control also had 400 ml of spring water to which 100 ml of water was added prior to the beginning of the trial to account for the possible effect of water movement on tadpole behaviour.

Cue-containing water was composed of what I assumed to be chemical compounds passively emitted from tadpoles. Cue-containing water was obtained by leaving experimental tadpoles in 200 ml of fresh spring water for 4 hours before the experiment started (round container, sized 10 × 7 cm). Then 100 ml of cue-containing water was added to experimental tadpoles in chemical and bimodal treatments in a reciprocal fashion. In other words, 100 ml of water from the chemical treatment was added to the bimodal treatment and vice versa. The final volume of each treatment pool was 500 ml.

Each trial was done individually. No other activities were carried out in the laboratory at the same time in order to avoid interruptions in the study. Laboratory lights were turned off and a separate lamp was used to obtain better quality videos.

2.3 Spatial video analysis

Following the activity analysis, I unexpectedly found that individuals from the vision treatment moved significantly more than other tadpoles. In a first attempt to understand why this might be, I decided to compare how the space use of vision treatment tadpoles differed from control tadpoles.

To find out whether tadpoles in the vision treatment differed in their space use from those in the control treatment, videos (Canon Legria HF R46, 25 fps) were further

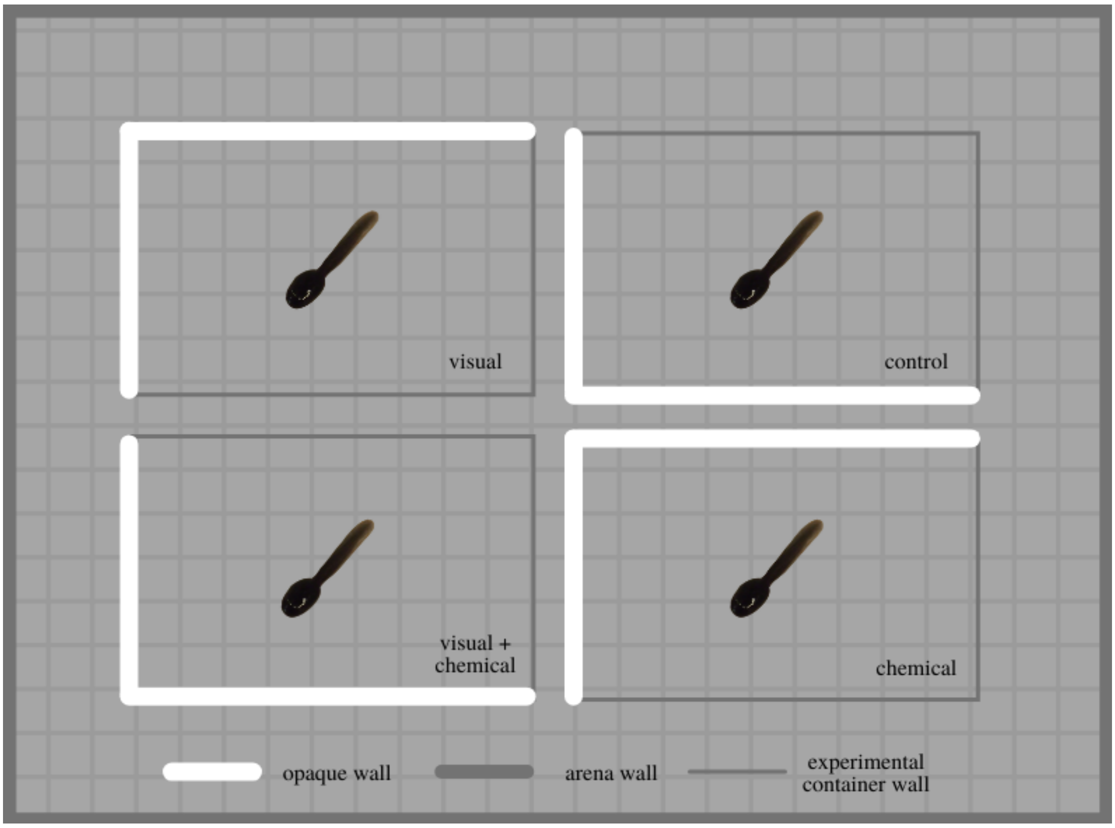

Figure 1. Trial setup. Large experimental arena was filled with tap water. Experimental containers within this arena were filled with fresh spring water. Each tadpole was exposed to one of four conditions: visual (the tadpole could see another tadpole through transparent plastic wall), chemical (the tadpole was exposed to water in which another tadpole had been, i.e., chemical cues), visual + chemical (the tadpole could both see and had water with chemical cues), and control (the tadpole in spring water). In each experimental container two walls were covered with opaque plastic, so that each tadpole was exposed to a different cue. The order of the cues in the figure is as follows - upper left: visual, upper right: control, down left: visual + chemical, down right: chemical.

analyzed in RStudio (v. 1.2.5033, RStudio Team 2019) with an object tracker program called trackR (Garnier 2021). Before running the analysis, origin and scale were set to centimeters.

2.4 Statistical analysis

2.4.1 Activity models

Models and statistical analysis were performed using the program R (v. 4.0.5 RStudio Team 2019) with the additional package “glmmTMB” (Magnusson et al. 2017). Point movements of activity (counts) were response variables used for analysis with family and clutch identity as random effects. Model fits and parameterization (i.e. interaction between predictors) were compared using Akaike Information Criterion (AIC, Akaike 1973). Models were built with “glmmTMB” (Magnusson et al. 2017) with a negative binomial family. Type of cue (four levels: visual, chemical, bimodal (both visual and chemical) and control (no cues)) and relatedness (two levels, non-siblings and siblings) were considered as model covariates. Residuals, overdispersion, and zero-inflation were checked and corrected using the package "DHARMa" (Hartig 2021).

2.4.2 Spatial models

Movement data from trackR were analysed with a spatial point pattern analysis using the package “spatstat” (Baddeley et al. 2015). Spatial information (Cartesian coordinates, x and y from video analysis) were converted into point pattern objects for further analysis. To remove spatial duplicates, points were subjected to an independent random displacement within a sensible default (rjitter). Spatial data was analysed using a Poisson model for multitype point processes (ppm) using maximum likelihood methods extended to multitype patterns.

Models considered tadpoles’ spatial intensity (based on spatial covariates), otherwise said as the proportion of time a coordinate was occupied, based on experimental treatment (called marks, which included vision and control treatments). Various intensity functions were considered (log-linear, log-cubic, log-quad) for model parameterization; based on model comparison methods using AIC, it was found found that a log-cubic function best described tadpole spatial intensity, as is frequently the case for spatially varying systems (Baddeley et al. 2015).

3 RESULTS

3.1 Activity levels

Compared to tadpoles in the control treatment, focal tadpoles in the vision treatment were significantly more active than tadpoles in the other experimental treatments (Table 1, Figure 2), regardless of whether the cues they were exposed to were from kin or from nonkin (Table 1). Activity of tadpoles in the chemical and bimodal treatments did not differ significantly from the control treatment (Table 1, Figure 2). No interactive effects between treatment and relatedness were found (see Appendix 1. Table 2). Tadpole activity did not differ between siblings and non-siblings (Table 1).

3.2 Space use

As tadpoles in the vision treatment were the only ones that showed higher levels of activity than tadpoles in the control treatment, I supplemented activity data with data on tadpole space use. Thus spatial point patterns of tadpoles were compared from vision and control groups to assess if the increased activity recorded in visual treatments could be explained by the patterns of tadpole displacement within the testing environment.

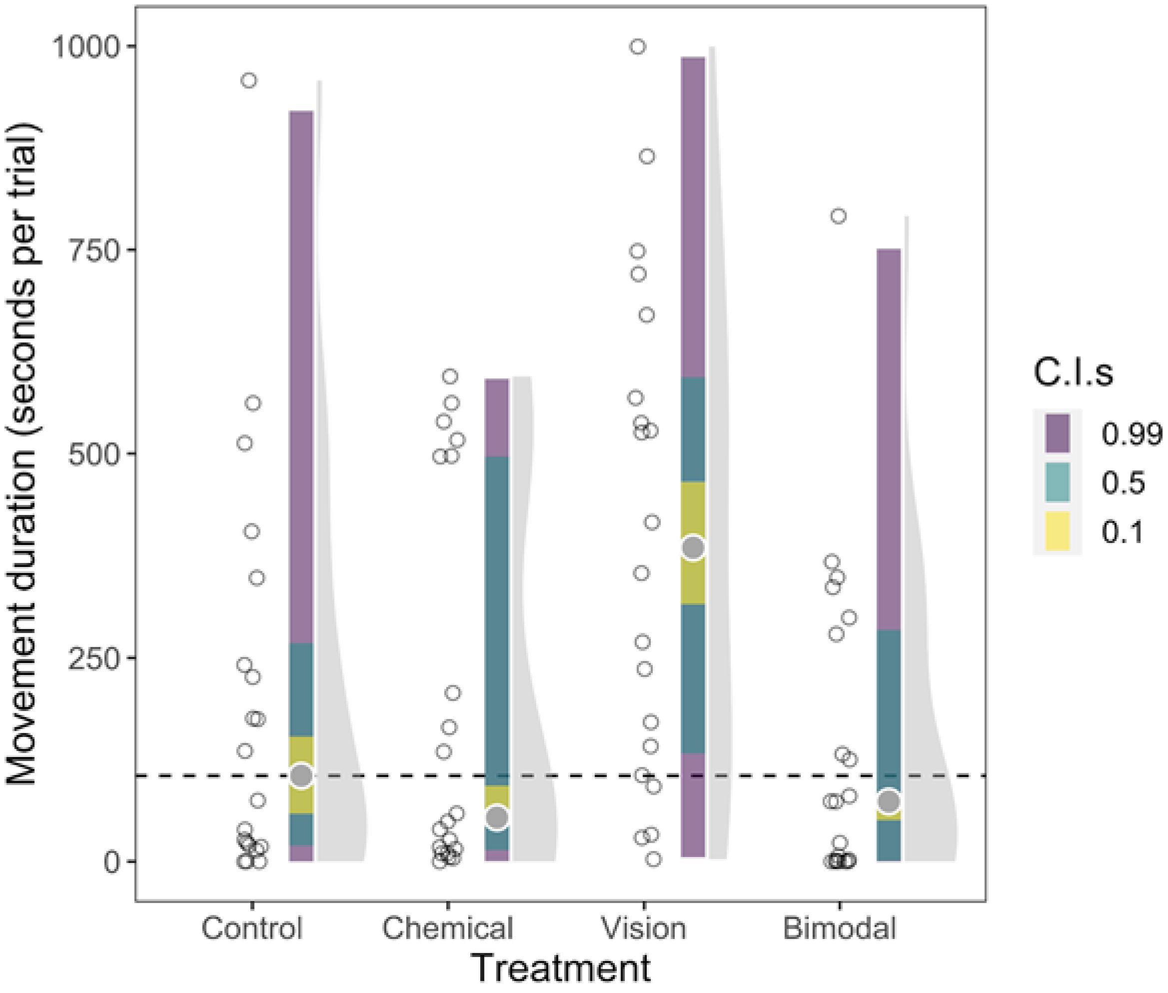

Figure 2. Experimental activity of tadpoles. Filled circles represent treatment medians. Dashed line is at the level of the control median (105.27 seconds) to simplify comparisons. Open points represent raw data. Banded columns represent treatment intervals at 0.1, 0.5, and 0.99 CIs. Half violins visualize the probability density function of each treatment. Tadpoles from the vision treatment move significantly more than other experimental conditions.

Table 1. Summary of negative binomial GLMM. Activity of a tadpole was predicted by the interaction between individual (visual, chemical, visual + chemical, control) and relatedness (two-level categorical variable). Family and clutch identity were accounted for as random effects, CI represents 95 % confidence interval. Intercept includes the control treatment and the non-sibling level of relatedness. σ^2 represents residual variance and τ represents random intercept variance.

Motion point events				
Predictors	*Incidence Rate Ratios*	*CI*	*Z stat*	*p*
(Intercept)	51.91	28.85–93.39	13.18	**<0,001**
Subject [Chemical]	0.84	0.46–1.53	0.57	0.566
Subject [Vision]	1.75	1.01–3.02	2.01	**0.045**
Subject [Bimodal]	0.64	0.35–1.19	-1.4	0.162
Relatedness [Sibs]	0.87	0.51–1.47	-0.52	0.601
Random Effects				
σ^2	0			
$\tau_{00Family}$	0.16			
$\tau_{00ClutchID}$	0			
N_{Family}	9			
N_{ClutchID}	31			

I hypothesized that tadpoles in the control treatment move randomly around their environment because they are not receiving any cues which would make them orientate to a specific direction. In comparison, I expected vision treatment tadpoles to spend more of their time at the clear container wall where they could see into another treatment. A higher intensity of occurrence by the shared container wall could mean vision treatment tadpoles attempting to gather visual information about the conspecific they are seeing.

The best fitting model was built with a relative risk that varied based on treatment type, meaning that the effects of treatment and spatial information interacted in predicting the overall space use of tadpoles (see AIC model comparison, Appendix 2. Table 3). Tadpole space use from both vision and control treatments were significantly different from each other (Nonstationary multitype Poisson process, Mark type: Vision, Estimate = -2.27, Zval = -49.10, see Appendix 3. Supplementary file). I detected an overall significant interaction effect in the spatial intensity model compared to an additive model (anova, df = 9, $p < 0.001$), where a likelihood ratio test confirmed that overall tadpole spatial positions were spatially segregated and depended significantly on the interaction between both location of the tadpole and treatment. Tadpoles from the vision treatment had significantly higher occupation intensities across repeated x/y Cartesian coordinates (Nonstationary multitype Poisson process, Zval = 336.7327), indicating a non-random occupation of space within the experimental arena. When these data are plotted, it can be seen that the vision treatment tadpoles had the highest level of occurrence by the shared container wall where they could see tadpoles from another treatment (Figure 3). In addition, tadpoles belonging to the control treatment had the highest intensity of space use in the corner of the experimental container, where the walls are covered with opaque plastic (Figure 3).

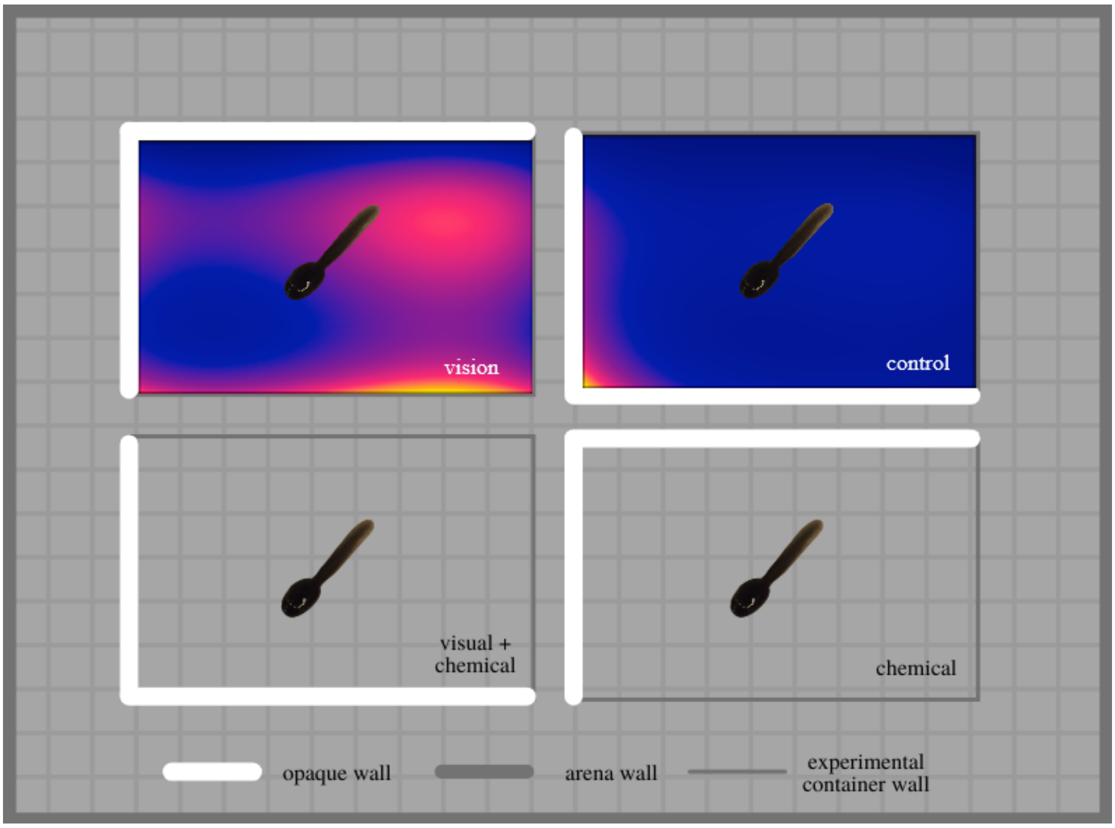

Figure 3. Tadpole space use within experimental context (see Figure 1 for full description). Average intensity calculated by points per cm^2. Warmer colors indicate a higher intensity of tadpole space use.

4 DISCUSSION

Effective communication plays a crucial role in the lives of animals, for example for attracting mates (e.g. extreme tail length in long-tailed widowbird: Andersson 1982), defending territory (e.g. roaring and parallel walking between red deer males: Clutton-Brock et al. 1979) or informing conspecifics of a threat posed by a predator (e.g. vervet monkeys alarm calls depending of approaching predator: Seyfarth et al. 1980).

Poison frogs are a group of animals with complex social behaviors. One example of this is their elaborate parental care. Some of the species can, for example, avoid depositing their tadpoles in water pools containing other cannibalistic tadpoles (Weygoldt 1980, Caldwell and De Araújo 1998, Summers 1999, Schulte and Lötters 2013). A previous study on *Oophaga pumilio* tadpoles showed that not only adults are able to communicate, but also tadpoles can: they can detect conspecific frogs by using multimodal cues (Stynoski and Noble 2012). However, to date not many studies have focused on communication between poison frog tadpoles. In this study, I quantified the activity of *D. tinctorius* tadpoles in response to cues stimulating different sensory modalities. The experiments included visual, chemical and bimodal (visual + chemical) cues. Contrary to my predictions, tadpoles exposed exclusively to visual cues were more active compared to the rest of the studied groups.

The increased activity in the visual treatment could have several implications for our understanding of tadpole communication from both cognitive and behavioural perspectives. Firstly, tadpoles might be better in motion detection than previously thought. Until this point, a study done about tadpole vision suggests that tadpoles are nearsighted with poor acuity (Mathis et al. 1988). However, that study was performed with tadpoles of *Anaxyrus americanus*, which differ from *D. tinctorius* tadpoles in many ways. For example, *A. americanus* tadpoles live in temperate regions, they develop in semi-permanent freshwater ponds or pools, and are nocturnal, whereas *D. tinctorius* is a diurnal tropical species whose tadpoles develop in ephemeral phytotelmata (Wright and Wright 1949, Rojas 2014, Rojas and Pašukonis 2019). In addition, *D. tinctorius* tadpoles are cannibalistic and benefit from a carnivorous diet; thus, as predators, they are dependent on good detection abilities (Rojas 2014, Fouilloux et al. 2020, in press). The importance of vision in predation has been suggested by an earlier field observation of tadpoles living in different water conditions: those in dark-tinted waters seem to be smaller than those in clear water despite the amount of potential prey being about the same (Bibiana Rojas, unpublished observation). This observation indicates that living in a visually challenging environment may influence the detection of prey and lowers hunting accuracy, hinting at an important role of vision in *D. tinctorius* tadpoles' survival. Considering this, *D. tinctorius* tadpoles seem to face very different selection pressures than *A. americanus* and thus, it is possible that *D. tinctorius*'s eyesight is better, or at least it could benefit from better vision.

Finding that tadpoles in the visual treatment were the most active differed from my original hypothesis. To better understand these unexpected results, I decided to further analyze whether tadpole movement was random or orientated towards a specific direction. My analysis of tadpole activity in the vision treatment supports the suggestion of the importance of vision to *D. tinctorius* tadpoles. When the space use of vision and control treatments were compared, tadpoles were significantly different from each other. As shown in figure 3, tadpoles in the vision treatment spent more time close to the open container wall through which they could see a tadpole, indicating that they are seeing their conspecific and maybe trying to figure out whether they are a threat. Also, tadpoles in the control treatment spend their time in the corner covered with opaque walls, which may indicate that they felt the safest there instead of the area exposed to the laboratory environment, thus indicating that they used information gathered through vision to choose their position in the container.

From a broader perspective, vision may be more important in communication of tropical diurnal tadpole species than previously thought. As mentioned earlier, Stynoski and Noble (2012) found in their study with *Oophaga pumilio* that tadpoles can detect an adult conspecific from a potential predator using visual cues. However, Kam and Yang (2002) studied tadpole-parent communication with another arboreal egg-fed species *Chirixalus eiffingeri*, but these tadpoles did not respond visually to a female frog. The difference between these two species could be explained by *Chirixalus eiffingeri* being nocturnal as opposed to *Oophaga pumilio*, which is diurnal like *D. tinctorius*. It would be beneficial for a diurnal animal to use visual cues for observing its surroundings. This possible trend should be studied to a wider extent with tropical tadpole species to gain better understanding of the importance of vision in tadpole communication.

Another possible explanation for increased activity of tadpoles in the vision treatment could be that myopic vision might not be a problem in a case where objects in fact are near to the tadpole, as in this study. Also, in a study performed by Hettyey et al. (2012) it was found that tadpoles of *Rana temporaria* reacted to the presence of predators not only when multimodal cues were available (chemical, visual, acoustic, hydraulic), but also when only visual cues were present, which is quite a surprising finding compared to earlier studies, in which visual cues were not found to play any significant role in tadpole antipredator behavior (Stauffer and Semlitsch 1993, Kiesecker et al. 1996). Hettyey et al. (2012) used smaller experimental chambers in their study than others in previous studies, which may be one reason explaining observed results about the use of visual cues when detecting predators. In smaller chambers, objects are closer to tadpoles, so despite tadpole near-sightedness, they can receive visual cues. In nature phytotelmata are small in volume (e.g., ranging from almost zero to 15.0 l in water holding capacity, Fouilloux et al. 2021), so a small experimental chamber size (0.5 l water in this study) mimics the natural situation better, i.e., prey items and predators are at close distances such that vision may provide immediate information on the position and potential attack distance of the threat.

The findings in this study show that the activity of tadpoles in the bimodal and chemical treatments did not differ significantly from the control treatment, which was unexpected. A possible reason why chemical cues did not elicit the predicted behavioural pattern could be explained by the experimental design: during the preparation of water samples used in experiments, tadpoles were not stressed, meaning that chemical cues (if any) in the water were passively emitted. Because tadpoles in the vision treatment behaved differently (increased activity) than tadpoles in the bimodal treatment, there may have been some information in the water of bimodal tadpoles which affected tadpole behavior. The only difference between visual and bimodal treatments were compounds dissolved in the water from tadpoles; ultimately, these differences suggest that the chemical cues may have contained information that the tadpole was not stressed or aggressive, so there was no need for the focal tadpole to increase activity. In comparison, tadpoles in vision treatment were lacking that chemical information, which made them search around the experimental container and orientate towards the tadpole they were seeing to gather more information. All in all, considering this, it would appear that tadpoles of *D. tinctorius* do use chemical cues to adjust their behavior, but in the case of this study, it is not seen as increased activity but staying still.

Future work modifying this study setup so that water samples come from pools where aggressive interactions had occurred would provide more information about the use and interpretation of chemical modalities involved in communication of *D. tinctorius* tadpoles. If tadpoles in olfaction and bimodal treatments show increased activity, it would indicate that they adjust their behavior according to chemical cues dissolved in water. On the other hand, if activity levels remain the same, and since we know that *D. tinctorius* tadpoles are aggressive based on previous studies (Rojas 2014, Fouilloux et al. 2020, in press), that would suggest that actual physical contact between individuals is needed to trigger aggression. Also, since I have established that space use differs based on treatment, which provides a possible explanation to the role of vision in tadpole activity, it would be interesting to analyze the space use in odor-based treatments as the continuation of this work.

5 CONCLUSIONS

In this study, I explored the sensory modalities that *Dendrobates tinctorius* tadpoles use to communicate with each other. I assessed communication modes by analyzing tadpole activity and space use when exposed to visual, chemical, or bimodal (visual + chemical) cues. Tadpoles in the visual treatment were the most active, and their space use was both non-random and differed significantly from control tadpoles. Vision treatment tadpoles had the most intense space use by their shared experimental wall, indicating that vision tadpoles were actively looking at other conspecifics with whom they could visually interact. I show here that vision is an important factor shaping tadpole behavior. This finding is unexpected, as until this point, most studies have assumed that tadpole vision is poor, and tadpoles mainly rely on chemical information in their communication. This study brings forth the importance of studying vision in tadpoles living in different habitats with different life strategies and serves as a ground for further studies on the selective pressures acting behind poison frog tadpole evolution.

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APPENDIX 1. Supplementary Table 1

Table 2. A table of a model that considers the interaction (*) between treatment and relatedness. The interaction between treatment and relatedness is not significant.

Motion point events

Predictors	*Incidence Rate Ratios*	*CI*	*Z stat*	*p*
(Intercept)	55.71	26.75–116.04	10.74	**<0.001**
Subject [Chemical]	0.76	0.30–1.96	-0.56	0.576
Subject [Vision]	1.42	0.59–3.39	0.78	**0.436**
Subject [Bimodal]	0.45	0.17-1.20	-1.6	0.110
Relatedness [Sibs]	0.81	0.31-2.07	-0.45	0.655
Subject [Chemical]* Relatedness [Sibs]	1.03	0.30–3.53	0.05	0.959
Subject [Vision]* Relatedness [Sibs]	1.30	0.42–4.01	0.46	0.644
Subject [Bimodal]* Relatedness [Sibs]	1.69	0.48–5.92	0.81	0.415
Random Effects				
σ^2	0.8			
$\tau_{00Family}$	0.24			
$\tau_{00ClutchID}$	0			
N_{Family}	9			
$N_{ClutchID}$	31			

APPENDIX 2. Supplementary Table 2

Table 3. AIC model selection for spatial varying intensity models, where smallest AIC value indicates best model fit.

Model	**Spatial fit**	**AIC**	**dAIC**
ppm(. ~ polynom(x, y, 3) * marks	**Log-cubic**	**-1053806**	**0**
ppm(. ~ polynom(x, y, 3) + marks	Log-cubic	-1005864	47942
ppm(. ~ polynom(x, y, 2) * marks	Log-quadratic	-1005407	48399
ppm(. ~ polynom(x, y, 2) + marks	Log-quadratic	-992326.6	61479.4

APPENDIX 3. Supplementary file

```
Point process model
Fitting    method:    maximum    likelihood    (logistic    regression
approximation)
Model was fitted using glm()
Algorithm converged
Call:
ppm.formula(Q = jitter_dat ~ polynom(x, y, 3) * marks, method = "logi")
Edge correction: “border”
     [border correction distance r = 0 ]
--------------------------------------------------------------------
--------------------
Quadrature scheme (logistic) = data + dummy
Data pattern:
Marked planar point pattern:  105650 points
Average intensity 639 points per square unit
Multitype:
        frequency proportion intensity
Control     52700      0.499       319
Vision      52900      0.501       320

Window: rectangle = [-0.2, 16] x [-0.2, 10] units
                    (16.2 x 10.2 units)
Window area = 165.24 square units


Dummy pattern:
(Stratified random dummy points, 660 x 660 grid of cells)
Marked planar point pattern:  435600 points
Average intensity 2640 points per square unit
Multitype:
        frequency proportion intensity
Control    217000      0.499      1310
Vision     218000      0.501      1320

Window: rectangle = [-0.2, 16] x [-0.2, 10] units
                    (16.2 x 10.2 units)
Window area = 165.24 square units
--------------------------------------------------------------------
--------------------
FITTED MODEL:

Nonstationary multitype Poisson process
Possible marks:
Control Vision
---- Intensity: ----

Log intensity: ~(x + y + I(x^2) + I(x * y) + I(y^2) + I(x^3) + I(x^2
* y) + I(x * y^2)
+ I(y^3)) * marks

Fitted trend coefficients:
          (Intercept)                      x                      y
         8.3453075137          -0.8674802197          -0.7829498588
              I(x^2)               I(x * y)                I(y^2)
         0.0799605292           0.0920850662           0.1550978220
```

```
                I(x^3)              I(x^2 * y)              I(x * y^2)
         -0.0023333615           -0.0026999920           -0.0050522181
                I(y^3)             marksVision           x:marksVision
         -0.0112261408           -2.3051672217            0.6866569264
         y:marksVision      I(x^2):marksVision     I(x * y):marksVision
         -0.3351442018           -0.0339538164           -0.1021012568
    I(y^2):marksVision      I(x^3):marksVision I(x^2 * y):marksVision
          0.1777792338            0.0002737027            0.0028859842
I(x * y^2):marksVision      I(y^3):marksVision
          0.0058535498           -0.0137524561

                             Estimate           S.E.          CI95.lo
CI95.hi Ztest
(Intercept)                  8.3453075137 2.157769e-02   8.303016e+00
8.3875990085   ***
x                          -0.8674802197 1.037137e-02 -8.878077e-01 -
0.8471527116   ***
y                          -0.7829498588 1.760079e-02 -8.174468e-01 -
0.7484529391   ***
I(x^2)                       0.0799605292 1.458133e-03   7.710264e-02
0.0828184167   ***
I(x * y)                     0.0920850662 1.873387e-03   8.841330e-02
0.0957568364   ***
I(y^2)                       0.1550978220 4.150947e-03   1.469621e-01
0.1632335293   ***
I(x^3)                     -0.0023333615 6.098641e-05 -2.452893e-03 -
0.0022138304   ***
I(x^2 * y)                 -0.0026999920 9.527412e-05 -2.886726e-03 -
0.0025132581   ***
I(x * y^2)                 -0.0050522181 1.550759e-04 -5.356161e-03 -
0.0047482749   ***
I(y^3)                     -0.0112261408 2.916116e-04 -1.179769e-02 -
0.0106545926   ***
marksVision                -2.3051672217 4.628384e-02 -2.395882e+00 -
2.2144525597   ***
x:marksVision                0.6866569264 1.721053e-02   6.529249e-01
0.7203889412   ***
y:marksVision              -0.3351442018 2.822338e-02 -3.904610e-01 -
0.2798273893   ***
I(x^2):marksVision         -0.0339538164 2.256759e-03 -3.837698e-02 -
0.0295306506   ***
I(x * y):marksVision       -0.1021012568 2.875495e-03 -1.077371e-01 -
0.0964653903   ***
I(y^2):marksVision           0.1777792338 6.138698e-03   1.657476e-01
0.1898108607   ***
I(x^3):marksVision           0.0002737027 9.152041e-05   9.432604e-05
0.0004530795    **
I(x^2 * y):marksVision      0.0028859842 1.353597e-04   2.620684e-03
0.0031512843   ***
I(x * y^2):marksVision      0.0058535498 2.307750e-04   5.401239e-03
0.0063058605   ***
I(y^3):marksVision         -0.0137524561 4.203060e-04 -1.457624e-02 -
0.0129286714   ***
                             Zval
(Intercept)             386.75630
x                       -83.64183
```

```
y                      -44.48378
I(x^2)                  54.83762
I(x * y)                49.15433
I(y^2)                  37.36444
I(x^3)                 -38.26035
I(x^2 * y)             -28.33919
I(x * y^2)             -32.57900
I(y^3)                 -38.49689
marksVision            -49.80501
x:marksVision           39.89749
y:marksVision          -11.87470
I(x^2):marksVision     -15.04539
I(x * y):marksVision   -35.50737
I(y^2):marksVision      28.96041
I(x^3):marksVision       2.99062
I(x^2 * y):marksVision  21.32085
I(x * y^2):marksVision  25.36475
I(y^3):marksVision     -32.72010

----------- gory details -----

Fitted regular parameters (theta):
           (Intercept)                      x                      y
          8.3453075137          -0.8674802197          -0.7829498588
                I(x^2)               I(x * y)                 I(y^2)
          0.0799605292           0.0920850662           0.1550978220
                I(x^3)             I(x^2 * y)             I(x * y^2)
         -0.0023333615          -0.0026999920          -0.0050522181
                I(y^3)            marksVision          x:marksVision
         -0.0112261408          -2.3051672217           0.6866569264
         y:marksVision     I(x^2):marksVision   I(x * y):marksVision
         -0.3351442018          -0.0339538164          -0.1021012568
    I(y^2):marksVision     I(x^3):marksVision I(x^2 * y):marksVision
          0.1777792338           0.0002737027           0.0028859842
I(x * y^2):marksVision     I(y^3):marksVision
          0.0058535498          -0.0137524561

Fitted exp(theta):
           (Intercept)                      x                      y
          4.210377e+03           4.200085e-01           4.570558e-01
                I(x^2)               I(x * y)                 I(y^2)
          1.083244e+00           1.096458e+00           1.167772e+00
                I(x^3)             I(x^2 * y)             I(x * y^2)
          9.976694e-01           9.973036e-01           9.949605e-01
                I(y^3)            marksVision          x:marksVision
          9.888366e-01           9.974212e-02           1.987062e+00
         y:marksVision     I(x^2):marksVision   I(x * y):marksVision
          7.152349e-01           9.666161e-01           9.029381e-01
    I(y^2):marksVision     I(x^3):marksVision I(x^2 * y):marksVision
          1.194562e+00           1.000274e+00           1.002890e+00
I(x * y^2):marksVision     I(y^3):marksVision
          1.005871e+00           9.863417e-01
```