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# Morphological response of the smooth newt to water color and its dispersal in managed forest landscape

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Amphibians are the most threatened group of vertebrates, facing multiple ecological stressors e.g. landscape degradation and habitat loss causing their rapid decline. Smooth newts (*Lissotriton vulgaris*) are semi-aquatic amphibians widely distributed in Europe. In landscapes newts have high demand of terrestrial and aquatic habitat types due to both habitats being essential to complete their life cycle. In boreal forests, forestry activities contribute to the decreasing number of wetlands, thus potentially affecting amphibian populations. In addition, water quality in the remaining wetlands is under multiple pressures: from eutrophication to the recently observed brownification phenomenon which is characterized by an increase in surface water color towards a yellow-brown hue. This latter process is mainly driven by increasing amount of dissolved organic matter (DOM) and dissolved iron (Fe) from terrestrial to aquatic environment and the declining deposit of atmospheric sulfur. Brownification has direct and indirect ecological effects on aquatic and semi-aquatic species. The attenuation of sunlight penetration by DOM affects e.g. primary production altering biological communities. While brownification may provide some benefits such as reducing harmful UV radiation, it can also pose challenges for species relying on visual cues, such as amphibians. Newts use visual cues for hunting and also in sexual selection. The effects of brownification to amphibians are not yet well understood, my thesis aims to partly fill up this gap of knowledge by investigating if the water color might drive morphological or colorimetric patterns of the smooth newts and addressing the effect of forest practices on newts dispersal. Data was gathered during summer 2023 in southern Finland. Passive sampling methods were used to trap smooth newts during the breeding season. Water color was measured at the different capture sites. The newts were brought to the laboratory to measure their morphological characteristics including size and colorimetric variables under anesthesia. The effects of forest practices were modeled by using MaxEnt and open-source data. Increase of water color was negatively correlated to Male newts Fulton index which indicates the newts body index. In addition, other responses varied between habitat types: male newts had less belly dots and also their tails hue peak wavelength in UV spectral range had positive relationship with water color in temporary wetlands but not in permanent. Whereas female newts belly hue in total spectral range had positive relationship with water color in temporary habitats but not in permanent. Forest age did not seem to contribute towards newts dispersal. Thus, newts respond more towards water color in temporary wetlands emphasizing the importance of these unique habitats for newts morphological diversity. More research is needed to fully understand the effects of brownification, especially during the early development of newts in the breeding ponds.

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Key words: Amphibian, Water color, Temporary wetlands, Coniferous forest, Habitat quality

# Table of contents

<b>1</b>	<b>Introduction .....</b>	<b>1</b>
1.1	<b>Brownification .....</b>	<b>2</b>
1.2	<b>Forest management, brownification and landscape scale habitat quality .....</b>	<b>4</b>
1.3	<b>Ecological effects of brownification on aquatic and semi-aquatic species .....</b>	<b>5</b>
1.4	<b>Aims and predictions of the thesis.....</b>	<b>6</b>
<b>2</b>	<b>Materials and Methods .....</b>	<b>8</b>
2.1	<b>Study area .....</b>	<b>8</b>
2.2	<b>Study species.....</b>	<b>9</b>
2.3	<b>Smooth newt capture.....</b>	<b>10</b>
2.4	<b>Smooth newt labwork.....</b>	<b>11</b>
2.5	<b>Image processing.....</b>	<b>12</b>
2.6	<b>Fulton index, dot patterns and selection of colorimetric variables .....</b>	<b>14</b>
2.7	<b>Water quality.....</b>	<b>15</b>
2.8	<b>Smooth newts suitable habitat during the breeding season at the landscape scale .....</b>	<b>16</b>
2.9	<b>Statistical analyzes .....</b>	<b>19</b>
<b>3</b>	<b>Results.....</b>	<b>21</b>
3.1	<b>Water quality.....</b>	<b>21</b>
3.2	<b>Smooth newts and brownification .....</b>	<b>22</b>
3.2.1	<b>Smooth newt body conditions.....</b>	<b>22</b>
3.2.2	<b>Smooth newt belly dot patterns.....</b>	<b>23</b>
3.2.3	<b>Smooth newt color response to water color.....</b>	<b>24</b>
3.3	<b>MaxEnt models.....</b>	<b>27</b>
<b>4</b>	<b>Discussion.....</b>	<b>30</b>
<b>5</b>	<b>Acknowledgments.....</b>	<b>40</b>
<b>6</b>	<b>References .....</b>	<b>41</b>
<b>7</b>	<b>Appendices .....</b>	<b>51</b>

## 1 Introduction

Amphibians are currently the most threatened vertebrate group, declining faster than mammals or birds (Stuart et al. 2004). The major factors driving the decline of amphibians are global climate change, habitat alteration (Stuart et al. 2004), overharvest (Winter et al. 2016) and decline of landscape quality (Holtmann et al. 2017; Calderon et al. 2019). Climate change affects amphibians' phenology, populations, distribution, and the interactions with other organisms (Li et al. 2013; Winter et al. 2016). Amphibians generally have poor dispersal abilities (Kovar et al. 2009). They suffer from high mortality rates when passing fields, roads or clear-cut areas (Cosentino et al. 2011). Hence stressors such as habitat loss, environmental degradation and habitat fragmentation have strong impact on their survival, their fitness and their population trajectories (Winter et al. 2016). Most amphibians live a semi-aquatic lifestyle, i.e. they require both aquatic and terrestrial habitats during their life cycle. Hence, natural wetlands (NW) are commonly used by amphibians to complete their full life cycle (Lehner & Döll 2004). Human activities are putting these habitats at risk: it has been estimated that over 70% of the world's wetlands have been destroyed (Kingsford et al. 2016) and the number of wetlands is still declining (Londe et al. 2022). The EU Water Framework Directive is generally not implemented to water bodies and wetlands of less than 50 ha. Hence, in October 2018, the RAMSAR Convention published the Resolution XIII alerting about the urgency of conservation and management of small wetlands. Among these wetlands, temporary wetlands have been largely overlooked (Kontula & Raunio 2018). Being temporary and typically small these temporary ecosystems are very fragile and extremely vulnerable to environmental change since they are mainly filled each spring by snowmelt or rain. Thus, changes in precipitation or temperature will affect their water level and quality in turn affecting their functioning and biodiversity (Lowe et al. 2015; Calhoun et al. 2017; Parra et al. 2021). These seasonal temporary wetlands are important breeding habitats for amphibians such as newts (Gómez-Rodríguez et al. 2009) due to the absence of predatory fish and the subsequent abundance of invertebrate prey (Compton et al. 2007).

Amphibian survival, breeding success and dispersal are affected by human practices such as forestry and road constructions. For example, forestry practices, e.g. clear cutting is known to affect the water quality and habitat connectivity of amphibians' terrestrial habitats and aquatic breeding sites. Vuorio et al. (2015) found that continuous forest canopy coverage and maintenance of sheltering vegetation close to the breeding pond is important for newts to be able to maintain their population. Hence, continuous forest canopy limits evapotranspiration

as compared to open fields (Cosentino et al. 2011), thus contributing to maintaining a certain level of humidity in the forested habitat which, in turn, is crucial for amphibians. In addition, humidity aids amphibians to gain better fitness, thus helping them to survive from potential predators (Rohr & Madison 2003).

Habitat connectivity is important for local amphibian populations (Gould et al. 2012), whereas disconnection of breeding site and suitable terrestrial habitat increase mortality especially for juveniles during emigration (Rothermel 2004). Newts of the genus *Triturus* have higher abundance when breeding pond and forest area are within radius of 400 meter in a crop plot dominated landscape (Joly et al. 2001). These studies support the importance of maintaining habitat connectivity between terrestrial and aquatic environment for amphibian distribution and abundance. Alongside connectivity, other factors such as water quality (e.g. water acidity (Leuven et al. 1986) and concentration of humic substances (Beebee 1987)) also drive the abundance and diversity of amphibians. In this context, the current surface water browning phenomenon is likely to impact amphibians and needs urgently to be addressed (Blanchet et al. 2022).

## 1.1 Brownification

During the past decades, surface water color has become darker in many aquatic environments. This process is known as browning or brownification. Brownification or water browning is the increase of surface water color towards yellow-brown hue. Humic substances the primary constituents of DOM (Sachse et al. 2005), absorb UV- and visible light contributing to the change of color towards yellow-brown. Dissolved iron (Fe) concentration also contributes to brownification (Kritzberg, & Ekström 2012; Weyhenmeyer et al. 2014). Brownification is mostly a result of the decreasing deposit of atmospheric sulfur (Futter et al. 2014; Pagano et al. 2014) and resulting increased transfer of dissolving organic matter (DOM) from the terrestrial to the aquatic environment (Evans et al. 2006; Blanchet et al. 2022). Atmospheric sulfur deposit resulting in the well-known “acid rain” peaked in 70’s and early 80’s (Mitchell et al. 2012) and started to decline in the 90’s (Monteith et al. 2007) due to stronger environmental regulations of sulfur dioxide, nitrogen oxides and greenhouse gases emissions. Decreased sulfur in the environment is strongly related to water color level of freshwater; soil – water DOC increases when  $\text{SO}_4\text{-S}$  concentration decreases (Ekström et al. 2011), therefore suggesting that decreasing acidification is being major driver of brownification (Evans et al. 2006; Driscoll et al. 2003). Browning is also dependent on seasonal variation, precipitation and temperature being most influential weather parameters

(Temnerud et al. 2014). In Finland precipitation and temperature are highest during spring and summer. This is important because higher precipitation is increasing the output of DOM to aquatic ecosystems (Corell et al. 2001). Global climate change is also contributing changes to both precipitation and temperature (Laudon et al. 2012; Weyhenmeyer et al. 2016), especially in the northern hemisphere where temperature increase is going to be higher than average. Climate change has potential to increase precipitation especially in northern parts of the earth, where brownification has often been observed, thus the increasing temperature will increase the run off from the terrestrial to the aquatic environment therefore contributing to water browning (Weyhenmeyer et al. 2014). Thus, on a global scale climate warming promotes browning in freshwater habitats (Evans et al. 2006; Larsen et al. 2011).

In natural freshwaters the absorbance is mainly influenced by Humic Substances and iron: water color is considered to be brown when DOC exceeds 10 mg/L (Blanchet et al. 2022), however the surface waters can be browner than the amount of DOM In the water (Erlandsson et al. 2008). Brownification is expected to impact all aquatic environments. Nevertheless, brownification studies have mainly focused on lakes (Kankaala et al. 2019) and rivers or streams (Sarkkola et al. 2009), and almost not at all on wetlands, including temporary wetlands despite their importance as biodiversity hotspots (Blanchet et al. 2022). Blanchet et al 2022 called for further research to understand brownifications role in different wetland types and at the landscape scale.

Brownification has larger biogeochemical consequences by altering carbon and nutrient cycles. Brownification is affecting the chemistry of aquatic environment by storing carbon and nutrients (de Wit et al. 2016) thus, possibly altering the amount of dissolved oxygen (DO) in water, which may lead to increased hypoxia (Krezminski et al. 2019). For amphibians DO is known to influence their larva development (Wassersug & Seibert 1975). Krezminski et al. (2019) stated that there is a possibility that brownification is a factor contributing to hypoxia and should be further studied in different habitat types. In addition, oxygen chemistry in water can be altered due to the possibility of brownification influencing how much dissolved oxygen concentration (DO%) is present in the water and, thus influence the community composition especially primary producers (Soulié et al. 2022) through food webs and effect trophic connectivity between predators and their prey (Ekau et al. 2010). DO can alter behavior or distribution of species, thus having ecological consequences. For example, DO effects zooplanktons vertical distribution, hatching success of copepods and predators' growth rates (Ekau et al. 2010) Nevertheless, Moriarty (2020) et al. found DO only having a weak

negative effect towards zooplankton. Thus, DO has the potential to have direct or indirect effects to smooth newts.

Brownification is increasingly studied in boreal forests in human managed or impacted landscapes, but more studies are needed to fully understand the factors causing this phenomenon. In addition, the impact of this process on biodiversity is still very poorly known (Blanchet et al. 2022). Amphibians are particularly interesting to study in this context since they depend on these browning aquatic environments and are sensitive to changes.

## **1.2 Forest management, brownification and landscape scale habitat quality**

Forest management in context of forest practices and land use are contributing to the browning of surface freshwater. For example, Nieminen et al. (2018a) suggested that changes in forest canopy cover can impact DOC and nutrient export, which affect brownification in boreal forest. Finér et al. (2021) estimated forest management to be responsible of 12% of organic carbon (OC) loading, in Finnish surface waters. It is still unsure how much forest management affects ecosystems OC therefore more research is needed (Härkönen et al. 2023).

In contrast to even aged forest management (EM), continuous cover forest management (CCF) maintains the continuum of forest canopy and is more heterogeneous habitat (Härkönen et al. 2023). In addition, mixed forests contribute less to the brownification as compared to coniferous forests (Kritzberg et al. 2020). Thus, compared to coniferous forests, mixed forest mitigating processes such as browning (Härkönen et al. 2023) are possibly more suitable habitats for species which are suffering from brownification.

Forest managements influences amphibians, for example DeMaynadier et al. (1995) found that clear cutting has generally negative effects to amphibian populations, especially to salamanders. While local extinctions caused by clear cutting are common, in the landscape scale extinctions are much less likely to occur due to clear cutting (Harper et al 2015).

Landscape is a mosaic of varying habitats where both the landscape and habitat quality features are known to have impact on amphibians (Holtmann et al. (2017). For example, Holtmann et al (2017) found that in urban landscapes stormwater ponds play an important role for amphibians. LoRusso et al. (2020) studied the effects of brownification in landscape context. They found that brownification in landscape is influenced by its features e.g. wetland drainage or streams subwatersheds, thus the browning process will vary between habitat types. Thus, for this reason brownification in landscape scale should be studied separately for different habitat types such as lakes, beaver sites, and temporary wetlands. Beaver sites were investigated separately from lakes due to beavers' influencing in the ecosystem e.g. altering

planktonic invertebrates and macroinvertebrates communities (Bashinskiy 2020), thus beaver lakes are different habitats than lakes. In addition, humans are not the only species engineering their environment. Beavers are also causing environmental changes that could possibly result in brownification. Blanchet et al. (2022) stated that beaver dams are increasing DOC concentration of the lake and their role in water quality should be included in research; especially in boreal landscape.

Human altered hydrological changes affect the fluctuations amount of DOC in forest waters. However hydrological changes are majorly affecting in short term fluctuation and cannot explain the ongoing long-term increase of DOC (Evans et al. 2006). Land use and hydrological changes can also alter how much Fe is present at the aquascape. Forest management and associated microclimate change of the soil surface can both alter the humidity of the ecosystem at the harvested patch level.

The effects of human activities to species distribution in the landscape can be modeled by using species distribution models (SDMs). SDMs were originally created to understand where the species can occur, and also to understand the species niche. For example, MaxEnt is an SDM designed to estimate the probability of species occurrence using the principle of maximum entropy. To model the species distribution MaxEnt relies on selected raster layers typically environmental variables, as predictor data and the species occurrence data. However, it can be applied to understand if the species are declining due to environmental issues such as forest practice. For example, Alakoski et al. (2020) in their article used MaxEnt to investigate the habitat selection differences between invasive and non-invasive beaver species in Finland including human influence in the model.

### **1.3 Ecological effects of brownification on aquatic and semi-aquatic species**

Brownification affects aquatic and semi-aquatic species and consequently the aquatic food web (Blanchet et al. 2022). In small ponds and lakes tailed amphibians are often top predators (Kuzmin 1991), which are affected by the flux of energy through the food web and regulating lower trophic levels. For example, paedomorphic species which undergo morphological changes during their life cycle such as newts, consume both macroinvertebrates and plankton. Bell (1975) found that smooth newts (*Lissotriton vulgaris*) larva firstly hunts passively consuming mostly zooplanktons (e.g. copepod and cladocera) but as the larva develops, they undergo change in hunting behavior towards more active hunting targeting macroinvertebrates such as larvae of chironomids. Thus, brownification by altering predation success or behavior of amphibians could possibly have wide ecological effects.



Water color is likely to influence the individual's behavior including sexual selection (Davenport 1992) since the female newts select their partner using visual clues especially in the UV spectral range (Secondi & Théry 2014). Male smooth newts' tail and crest have an important role in sexual behavior of smooth newts. Tail and crest traits are highly visible sexual dimorphs of smooth newts during the breeding season (Halliday 1974; Green 1989) thus in order for the males to display themselves I predicted that they would invest more or less resources into secondary sexual characters when water color is darker and the also when habitat lacks predation. Sexual dimorphism has long been studied due to the highly differing traits between species sexes (Darwin 1871, Dayan & Simberloff 1994). Displaying higher quality of sexual dimorphic traits is often predicted to be beneficial in mating, and correlates with resource availability or how much extra resources the individual has to spend to secondary sexual traits (Bonduriansky 2007). Hence, it could be possible that brownification would drive direct or indirect sex specific responses.

Predation prediction is based on antipredation behavior or the predation risk allocation hypothesis demonstrating animals change in resource allocation due to predation risk levels (Lima & Bednekoff 1999).

Secondi et al. (2012) found that smooth newts use UV-reflecting mating signals. For this reason, males could invest resources in body color to make themselves more attractive; the males color attractiveness and body condition provides information for females from the male as a possible mating partner. Smooth newt females also spend more time close to males when UV light and signals are visible (Secondi et al. 2012), therefore brownification could possibly affect sexual selection. The lack of UV-light also makes it harder for newts to recognize species of their own type from close related species (Secondi & Théry 2014).

Despite the possible issues caused by brownification, to some extent water browning could have positive effects for some species, for example by decreasing UV-radiation, especially UV-B (290-320 nm) that is biologically the most harmful wavelength (Palen et al. 2005). UV-B is also harmful for many species, especially for amphibians by affecting eggs hatchability and larva development via damages at cellular level (Blaustein et al. 2005).

#### **1.4 Aims and predictions of the thesis**

In this project, I aim to unravel if smooth newts might be affected by the brownification process. Since no long-term data set exists to assess the effect of brownification on amphibians, I will focus on the effect of different water color on smooth newts coloration and their body condition. In addition, I aim to reveal if brownification affects DO% in the

breeding sites and thus, could have ecological consequences for newts. More specifically, I will investigate if individual body conditions differ in the landscape scale depending on the suitability of the habitat in context of water color and habitat type. Habitat type being under my interest due to the vulnerability of temporary wetlands and their importance as breeding sites. I will assess if individuals invest in brighter color in darker environment to increase the contrast of color as the breeding environments color changes, or on the opposite if they exhibit duller colors in brown environment as a result of change in prey availability or loss of selective pressure (female not being able to use visual clues to assess mate quality). I will investigate if newt's color and body condition vary with water color. I will investigate the suitable habitat of smooth newts during breeding season inspecting environmental variables which may limit their dispersal or impact their choice of breeding site. I will then discuss if the selected landscape variables and water color have correlation and if they have possible impact to smooth newts characteristics. Forest practices are expected to favor water browning, to hinder movement of newts, and thus to affect their presence in wetlands at the landscape scale.

## 2 Materials and Methods

### 2.1 Study area

The Evo study site is located in southern Finland (61°120 N, 25°070E, Figure 1). Most of the wetlands used for the study are located in Evo Natura-2000 area (FI0325001). Evo Natura zone is located at Hämeenlinna, Padasjoki and Asikkala. Evo Natura-2000 area was established in 1998 and is a habitat directive site having a total land area of 7860 ha. The area is mainly state-owned (Metsähallitus). The landscape is mostly constituted of exploited coniferous forest and mixed coniferous-deciduous forest. One hundred and thirty-six lakes are found in the area. The landscape is largely influenced by forest management for timber production.

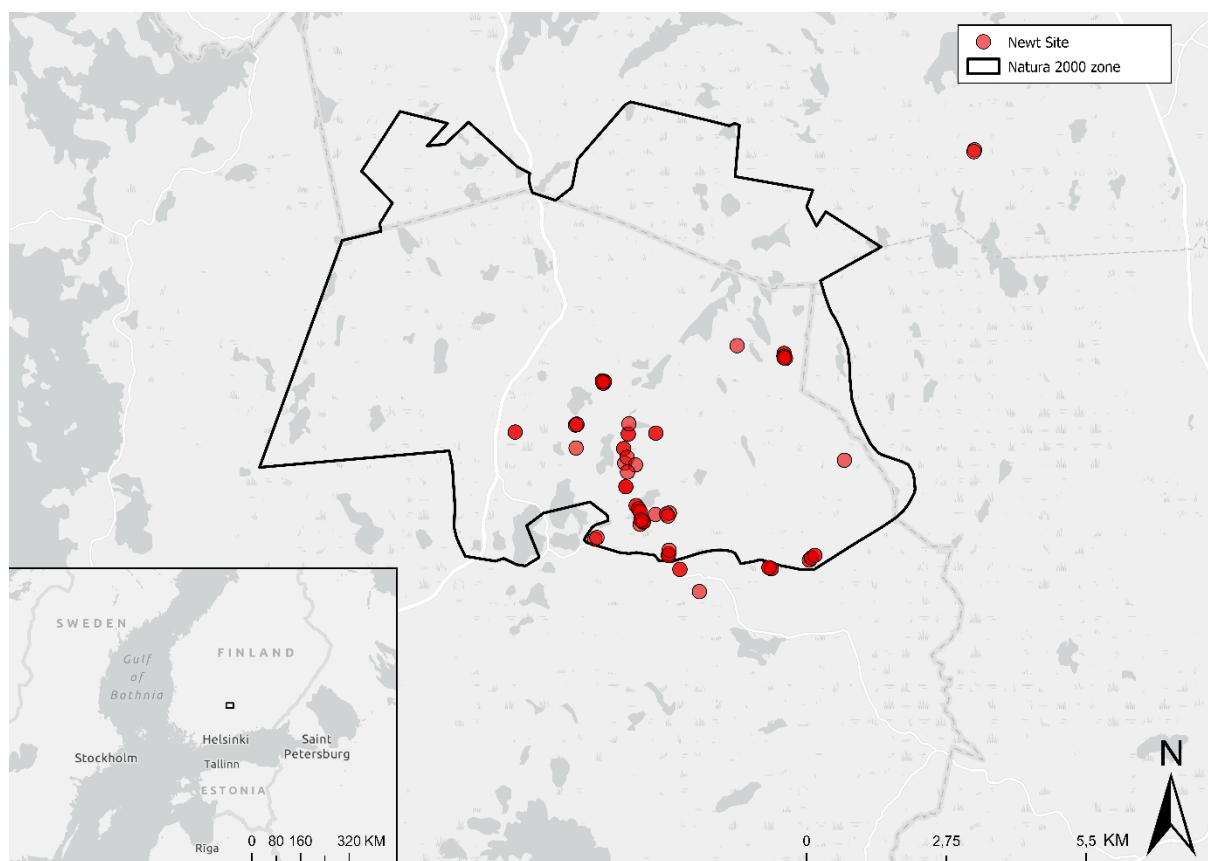


Figure 1. Study site map and natura 2000 zone in EVO, Finland, and all sites where newts have been captured or observed from 2021 to 2023 year.

In the study area, 42 possible smooth newt breeding sites were chosen to be trapped to capture adult smooth newts including permanent (lakes and beaver sites) and temporary wetlands. Site selection was based on previous sampling of the area by the supervising team and collaborators in the area.

Like all other amphibian species in Europe the smooth newt is a protected species. The capture and the experiments took place in accordance with the permits granted by the Finnish authorities, Ely-keskus and ESAVI (ESAVI/6410/2020), under the supervisors Céline Arzel, Clarisse Blanchet and Jean Secondi. All the supervisors have been granted certification of FELASA a, b, d or equivalent. Metsähallitus, HAMK, UPM metsä and city of Hämeenlinna have granted permission to collect samples on their respective land.

## 2.2 Study species

Smooth newts are small generalist carnivorous semi-aquatic amphibians and belong to the family of *Salamandridae*, and the subfamily of *Pleurodelinae* (newts). Among all the genus of *salamandridae* the newts are only species living a semi-aquatic lifestyle. Adult newts are primarily active during darkness in early mornings and late evenings (AmphibiaWeb 2024). This applies for both aquatic and terrestrial phases of life, however they can also be active on land during daylight when it is rainy (AmphibiaWeb 2024). Smooth newts undergo metamorphosis during their life cycle from aquatic larva phase to terrestrial eft (juvenile) phase. In addition, their life includes an adult phase which includes both terrestrial and aquatic lifestyle. Smooth newt juvenile and adult newts live after metamorphosis outside the breeding periods. Smooth newts reach maturity between the ages of three to seven years old (Bell 1977). The breeding season is from March to early July. In Finland, the breeding starts from late April to early May depending on when the ice cover has melted (Vuorio et al. 2015). Smooth newts spend varying time in the breeding pond for up to 6 months, suggesting that some of newts stay in the pond to gain weight for overwintering (Verrell & Halliday 1985). Adult smooth newts return to same breeding site every year whereas the juveniles when reaching maturity disperse to close ponds (Bell 1977). However, in our study site where summer is short and especially in most of our observed sites in evo are short term, temporary ponds they spend less time in the breeding site.

Smooth newts can be found in most of Europe in humid and aquatic areas (Sinsch et al. 2018), in Northern Europe they are most commonly found in coniferous landscape (Skei et al. 2006). In Finland smooth newts are often found in natural wetlands that are ideal for newts who have semi-aquatic life cycle spending most of their life in land. The newts prefer small fishless ponds, such as temporary wetlands (Griffiths 1997), with relatively high level of vegetation as a breeding site (Beebee 1981; Skei et al. 2006). However, smooth newts also breed in lakes (Vuorio 2016), in which predator fishes are more likely to be present. In those cases dense vegetation offers hiding spots and thus increases the survival of smooth newts

(Dolmen 1976). Forest dominated landscapes are the most suitable environment for newts in boreal zones offering more resources compared to open, boggy surroundings including shelter from predation, high abundance of food and sites to hibernate (Skei et al. 2006).

Smooth newt females and males mature at the same age. They keep growing every year (Bell 1977), nevertheless body size is a poor indicator of smooth newts age. Halliday & Verrell (1988) pointed out that smooth newt's snout-vent length (SVL) growth rates are similar for both sexes despite the similar growth rates of adult newts body their size vary significantly within even if the newts are same age. The size of adult smooth newt ranges between 7 to 11 cm, half of the size being tail and weight ranges from 1.5 to 4 grams. Smooth newts have marked sexual dimorphism which is important for sexual selection among the smooth newts. During the breeding season males develop secondary sexual characters including high dorsal crest, pattern of dark spots and yellow-orange stripes on the ventral area and base of the tail. Individuals have unique patterns, which are used for sexual signaling. These male traits and males' behavior including three movements with tail (the wave, whip, and the fan) are involved in the female's selection of mating partner (Halliday 1975).

Smooth newt phenotypes and subspecies respond to environmental conditions (Bell 1977), thus water color and diet could possibly impact smooth newt coloration and other sexual dimorphs and eventually sexual selection.

### 2.3 Smooth newt capture

The smooth newt trapping took place from 15.05.2023 to 27.06.2023 during the breeding season of smooth newt. Passive sampling technique was used for trapping. Three different types of traps were used for two purposes.

1. Bottle traps and fish nets were used specifically for newts.
2. Activity traps were used for capturing primarily invertebrates but smooth newts inside the traps were also included in the study.

Newt bottle traps were made from 2L plastic bottles. A quarter of the bottle was cut off from top of the bottle. The cut off top was placed back invertedly, thus creating a funnel which encourages the newts to enter the bottle trap. The size of the fish traps was 46 x 22 x 22 cm (Length x width x height). Activity traps consist of a plastic funnel attached to a 0.75 or 1-liter glass jar using wire. The outlets (widest part of the funnel and out of the jar) measure 125mm and the inlets (narrowest part of the funnel and in the jar) measure 25mm. The traps

were placed horizontally below the water surface without bait to capture newts and both benthic and pelagic invertebrates (Hyvönen & Nummi 2000). An air bubble was left in each trap to make sure that captured newts had access to oxygen.

At each site we placed 10 activity traps, 20-30 newt bottle traps and five fish nets. The traps were set to three sites (once at two sites) every Monday and Wednesday and retrieved on the next morning. Captured newts were placed in a bucket containing water from the location of capture. The newts were sexed and a maximum of 30 newts from each site were brought to Lammi biological station for measurements.

## 2.4 Smooth newt labwork

In the lab, smooth newts were anaesthetised with a solution at 0.2g/l concentration of MS-222 and the measurements were taken while the newts were motionless.

Smooth newts' morphological measurements were taken from two positions: ventral and lateral by taking pictures with a Nikon D3400 camera. ImageJ version 1.54d was used to analyze the body and tail length, snout-vent distance (SVL), size of the crest and quantify belly dot patterns (Figure 2). ImageJ software uses pixels from the picture to measure the size, thus a millimetric paper was used to transform the pixels into a metric system.

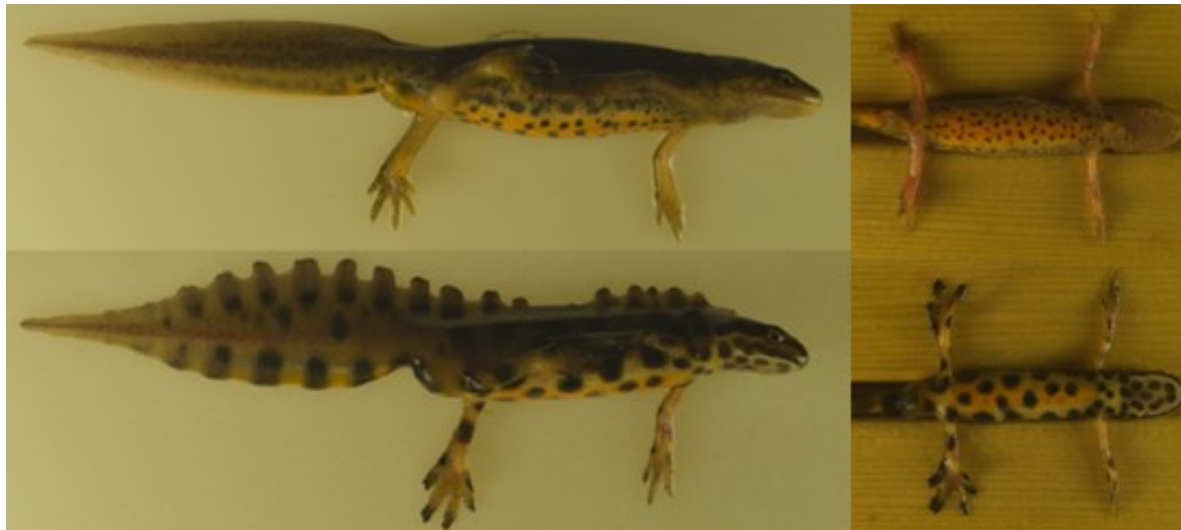


Figure 2. Lateral and dorsal pictures of smooth newt female (top) and male (bottom).

A spectrophotometer AvaSpec-2048 combined with a light source AvaLight-DHS was used to measure smooth newts' belly, back color, tail's white part and caudal part behind the cloaca by using reflectance spectra 300-700 nm. Measuring quantities of mean brightness (amount of reflected light), hue (dominant wavelength) and chroma (dispersion of wavelengths) at UV range from 300 to 400 nm and visible 401 to 700 nm. UV and visible light spectrum were

analyzed separately and together. I used three colorimetric measurements: Brightness, hue, chroma. Thereby we measured reflectance ( $R, \lambda$ ), the proportion of light reflected by a surface at each wavelength. The measurements were done while the animals were wet at a measurement angle of 45° degree. Colorimetric measurements were doubled for each body part of all individuals and averaged for analysis.

Smooth newts were weighed (g) with a Mettler PC 4400 scale, and then DNA samples were taken by gently rolling cotton tubs in the newts' mouth. The head of the cotton tub was placed in an Eppendorf tube filled with ethanol.

After all the measurements were done, the newts were placed in a 10x7x5 cm plastic box containing water from their natural habitat to wake up. Then newts were placed back in buckets containing water from their natural habitat in a climate control room overnight. They were released back to their site of capture the next morning.

## 2.5 Image processing

The software ImageJ calculates length and size according to the number of pixels in the picture. To minimize the differences between pictures taken from the Smooth newts', the pictures were edited to match each other. I used a surface reference of 1 cm<sup>2</sup> by setting a 2cm long and 0.5cm wide polygon inside the millimetric paper for each picture (Figure 3). Then I made a polygon of the belly and measured it. I recorded the number of pixels for polygons, reference, and belly (area). Bioimage analysis was used to detect belly dots by using the threshold dialog to calculate the dot pattern. In threshold dialog I used settings triangle and red as color. after which I used a particle analysis tool to calculate the amount and area of the dots (Figure 4).

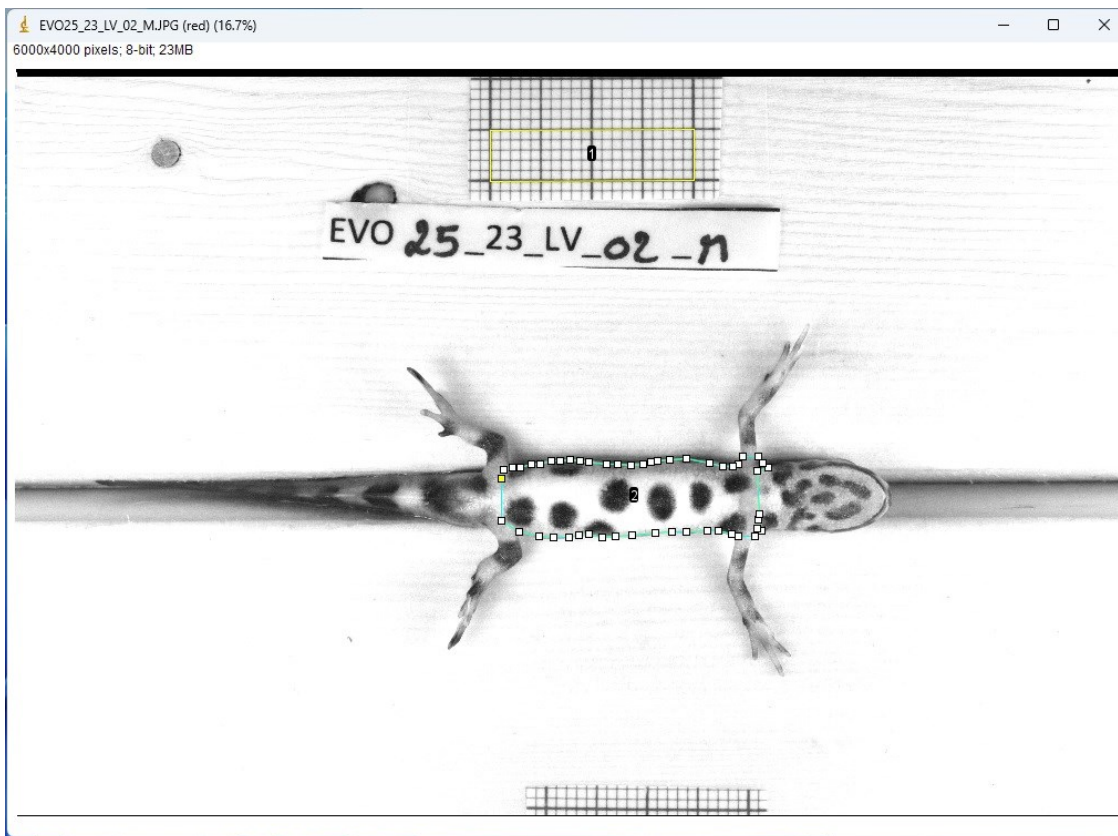


Figure 3. Picture of a smooth newt as processed in the ImageJ software. The picture includes the 1 cm<sup>2</sup> reference at the top of the picture (1) in this example, the code of the individual (EVO 25\_23\_LV (*Lissotriton vulgaris*)\_02\_M), the smooth newt with the polygon for the belly (2).

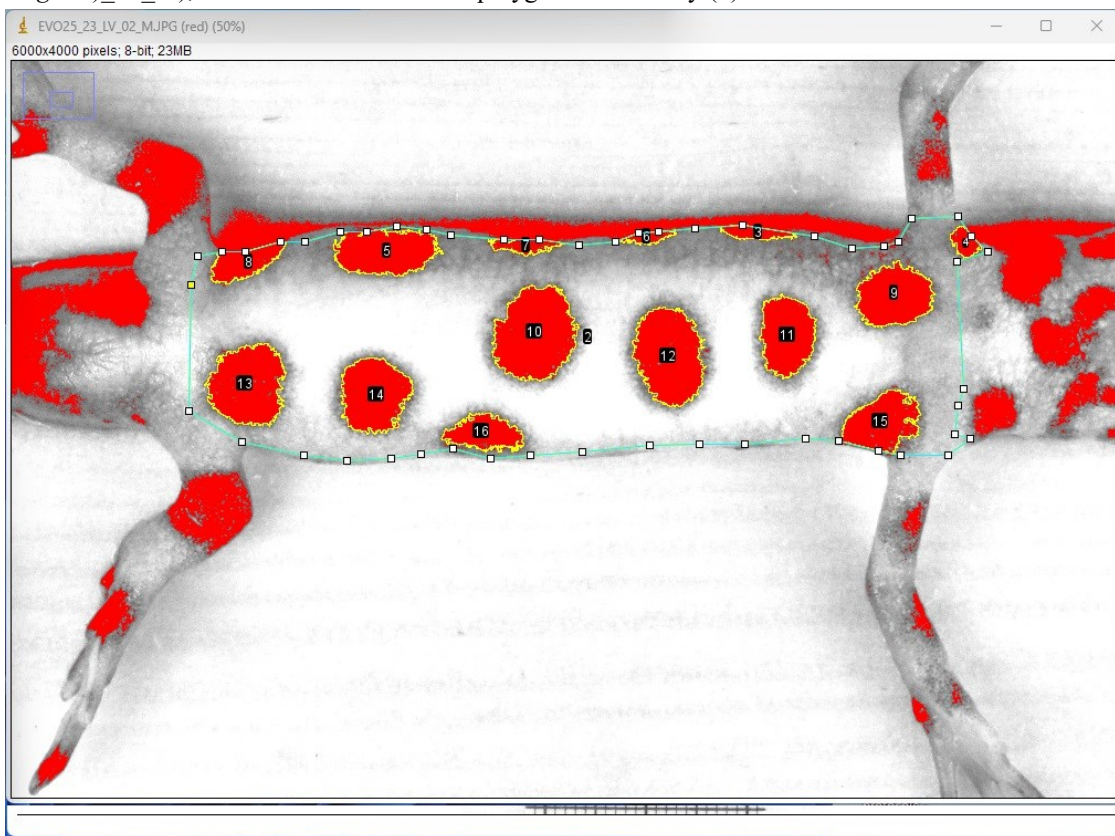


Figure 4. Example of the measurement of the number of belly dot patterns of a Smooth newt. In this case 16 dots are identified by the software ImageJ.



## 2.6 Fulton index, dot patterns and selection of colorimetric variables

Fulton index is a metric informing the relative weight of an individual compared to its length. In Fulton index a high value typically refers to an individual in better condition as compared to individuals with lower values. In this thesis it measures the body condition whereas SVL measures the body size, thus in order to make it independent from body size the SVL was calculated in cube. In addition, it is a very good way to measure Fulton index in animals with isometric growth e.g. newts. In the data Fulton index had following formula:

$$\text{Fulton index} = \text{Weight} / \text{SVL}^3$$

Six variables defined the belly dot patterns: the number of dots, the total surface area of dots, the circularity of the dots, the solidity of the dots, the size of the dots (cm) and the density of dots on the belly surface. The total surface area (TSA) of dot was calculated as:

$$\text{TSA} = \text{Number of Pixels} \times (\text{Pixel Width}) \times (\text{Pixel Height})$$

In Circularity formula A stands for the area of the dot, P is the perimeter of the dot. Circularity vary between 0 and 1 and in ImageJ it is calculated with following formula:

$$\text{Circularity} = (4 * \pi * A) / P^2$$

Lastly the solidity of the dots is calculated from the belly area and the area of the convex hull also called convex closure or convex envelope (area of the smallest convex polygon that entirely encloses in the object). Like circularity the solidity also varies between 0 and 1. The formula of the solidity:

$$\text{Solidity} = \text{Area of belly} / \text{Area of Convex Hull}$$

Circularity of the dots highly correlated with the solidity (males corr = 0.859, females corr = 0.856). Size of the ventral dots highly correlated with males total surface area of dots in the newts belly (0.798) and females number of dots (-0.657). Thus, both circularity and size of the ventral dots were removed. All models including belly dot variables were modeled using LMM with gaussian distribution.

Reflectance brightness, chroma and hue were used as colorimetric parameters (Table 1, see <https://rafaelmaia.net/pavo/reference/summary.rspec.html>). These colorimetric variables were chosen due to previous understanding of smooth newts' sexual selection: brightness and chroma often indicates the individual's fitness of the newt, brighter and more intensive (vivid) males being more attractive for the females and also hue was used due to the bright orange color in the belly (de Solan et al. 2022; Lüdtké & Foerster, 2018; Secondi & Théry, 2014). Overall, they are colorimeters used in sexual selection.

I chose mean brightness (B2), spectral purity (intensity or vividness of color, S4), contrast (difference in hue and saturation, S6) and the peak wavelength of hue (H1) for UV range and B2, S4, S6, chroma (S8), wavelength at bmaxned of hue (H2) and wavelength at Rmid of hue (H3) for visual range and all of them for total spectra. After the selection a correlation matrix was run, sex specifically and separately for smooth newts' tail and belly. For both sexes tails total range spectrum B2 highly correlated with S6 (males corr = 0.903, females corr = 0.855) and males total tail S8 with H3 (males corr = 0.824) S8 with S6 (0.767). In addition, I found high correlation in both male newts belly's total range spectrums H3 to have strong correlation with S8 (0.824). Due to these high correlations, I removed S6 and H3 from total range of spectrum analysis. In UV range for both sexes tails B2 highly correlated with S6 (males corr = 0.806, females corr = 0.913) and with S4 (males corr = 0.783, females corr = 0.910). Here to reduce the number of analyzes both S6 and S4 were removed whereas B2 was kept instead of analyzing both S4 and S6. For visual spectrum both sexes belly S6 and B2 highly correlated males corr = 0.923, females corr = 0.959) and belly S8 with H3 (males corr = 0.810, females corr = 0.670). In addition, males tails visual range spectrum S8 highly correlated S6 (males corr = 0.730). S6, H3 and S8 were removed from visual range analyzes.

Table 1. Description of used colorimetric variables. Note that in H2 the bmaxneg stands for maximum negative wavelength (value) and Rmid in H2 refers to the midpoint of wavelength (color value).

Colorimetric variables	Description
B2	Mean brightness
S4	Spectral purity
S6	Contrast
S8	Chroma
H1	Peak wavelength, hue
H2	Wavelength at bmaxneg, hue
H3	Wavelength at Rmid, hue

## 2.7 Water quality

Water samples were collected at the study locations and kept in the dark in a cool box packed with ice up until arrival at Lammi Biological station (LBS) within a few hours after collection. Samples were then placed in a fridge until analyses which were conducted by LBS within less than 48h after sample collection. Water color is measured in milligrams of

platinum per liter (mg Pt/l) at 410 nm spectra (ISO7887). In site I used an optical dissolved oxygen (DO) and temperature instrument (YSI ProOD) to measure water DO % and DO Mg/L.

## 2.8 Smooth newts suitable habitat during the breeding season at the landscape scale

For species distribution prediction model (SDM), I chose environmental variables which are known to be important for newts. Smooth newts are sensitive to forest age, type, and landscape wetness (Vuorio et al. 2015). In addition, newts have poor dispersal abilities thus, the amount of differences in topographic elevation (Terrain Ruggedness Index, TRI) could be a possible dispersal barrier.

Thus, the following environmental predictor variables were extracted:

1. Topographic variables: terrain ruggedness and topographic wetness index (TWI) as an indicator of humidity.
2. Terrestrial habitat variables: forest stand age categories and proportion of forest types
3. Aquatic habitat variables: Permanent water bodies, edge density for ditches, and mean proportion of time steps with detected water in non-permanent water bodies

My primary GIS data is from two open access data sources including topographic data from national land survey of Finland and environmental data from Finnish environmental institute and Finnish Forest Center (<https://www.metsakeskus.fi/fi/avoim-metsa-jaluontotieto/aineistot-paikkatieto-ohjelmille/paikkatietoaineistot>) and <https://kartta.paikkatietoikkuna.fi/>). Newts' presence data is based on observations and captures of newts from year 2021 to 2023 as part of the POOL project surveys (see <https://sites.utu.fi/pool/>). I used ArcGIS Pro 3.1.0 version to create the different layers used in this study.

I used a 2m resolution digital elevation model (DEM) to make the TWI and the terrain ruggedness index (TRI). In order to process the TWI, I used ArcGIS Pro's tools in the following order: spatial analysis tool fill DEM -> hydrology analysis flow direction from fill\_DEM -> hydrology spatial tool flow accumulation and then scaled with a raster calculator. Then I used surface analysis spatial tool for slope in degree for Fill\_DEM. The slope was used to process a radiance of slope using a map algebra raster calculator and then the radiance of slope was processed to tan slope using raster calculator tool. I then used these

new layers to create TWI using a raster calculator. To process a TRI, I used spatial analyst focal statistic tools to create mean, maximum and minimum. I then used spatial analyst tool raster calculator to process them into a TRI.

I created a raster for permanent waters including rivers, river lines and lakes. I used a water summary analysis to determine if the area (pixel) had been under water during the breeding season.

I used the forest data to create layers for four different patch ages and four tree patch types. Ages layers included following:

1. <2 years after cut. Mostly low vegetation
2. Between 2-10 years, tree vegetation does not dominate and there is a denser understory.
3. Between 10-20 years, tree canopy cover is not complete
4. > 20 years canopy is complete, and litter has accumulated

The forest patch types were chosen as following: 1. Pine, 2. Spruce and 3. Deciduous forests and 4. coniferous tree.

All environmental data was rescaled to 5m resolution. However, using such a high resolution is not exactly good for modeling amphibian's species distribution. In amphibian studies higher resolution is often used when predicting suitable habitat e.g. for conservation purposes. For example, 1 x 1 km resolution was used in national scale studies for Lao newts (*Laotriton laoensis*) (Chunco et al. 2013) and southern crested newt (*Triturus karelinii*) (Pourhallaji et al. 2021). In another study by Vaissi et al (2019) they used a 30m resolution for yellow spotted mountain newt (*Neurergus derjugini*). Compared to the study conducted by Vaissi et al. (2019) the study site in this thesis was done in a much smaller area with less difference e.g. in elevation thus I chose to set the first resolution of study to 50m.

Finally, I normalized all values to vary between 0 – 1 and created fishnet (grid cells) for four different sizes 50m, 100m, 200m and 300m resolutions. Different resolutions were created and tested to understand how the scaling impacted the results. After normalizing and creating the fishnets I used zonal statistics to calculate the values inside each grid cell in the fishnet. For TWI and TRI, I calculate the mean values for each grid cell and for rest of the data I calculated the proportion of the raster values in each grid cell (see figure 5 as an example of the raster's after zonal statistics).

I used 77 unique newt breeding sites in the landscape as occurrence points together with environmental predictors in the MaxEnt model.

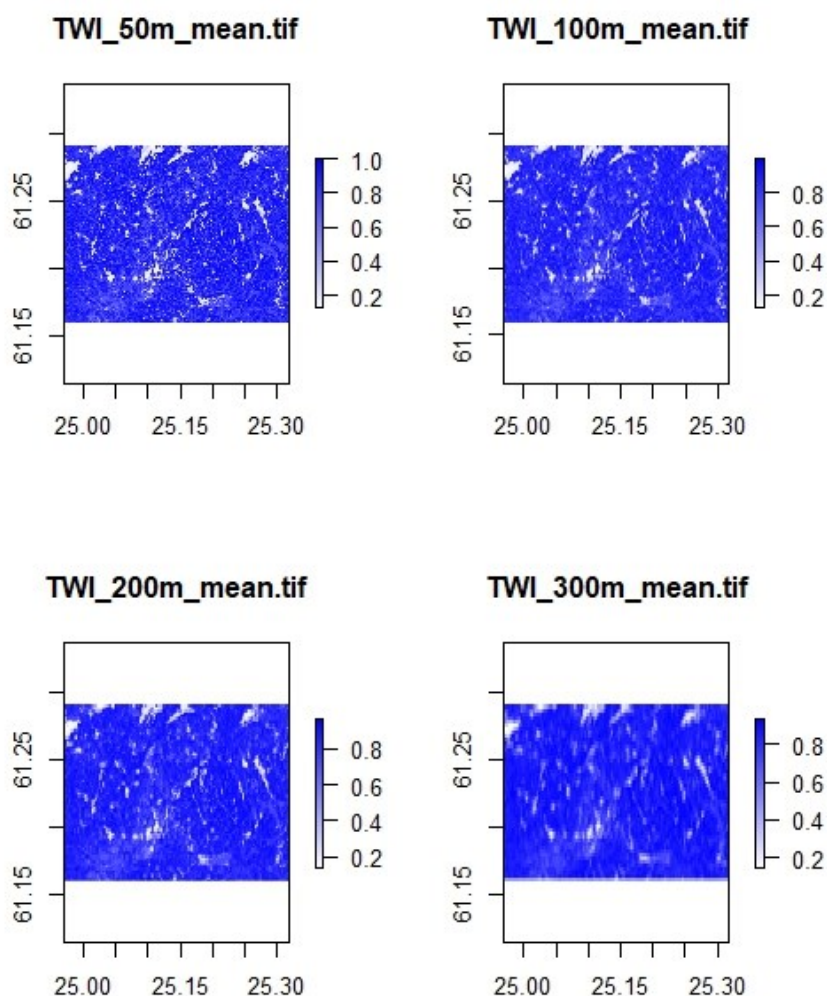


Figure 5. TWI as an example of raster layers in all resolutions including 50m, 100m, 200m and 300m. The value was normalized vary between 0 and 1 in each grid cell.

Together with the smooth newt's occurrence data I used the environmental variables to make a species distribution model (SDM) based on the principle of maximum entropy (MaxEnt). MaxEnt is a correlative model based on machine learning and built on the premise that variables correlate with another in a predictable manner.

MaxEnt was used to investigate if newt occurrence correlated with forest practices with the hypotheses that forestry practices hinder the dispersal and then occurrence of smooth newts in the study area.

## 2.9 Statistical analyzes

Statistical analyzes were done with Rstudio version 4.3.1 (R, core team 2022). Lme4 package (Bates et al. 2015) was used in all linear mixed models (LMMs) and MASS package (Zuur 2009 et al. 2009) for generalized linear mixed models (GLMMs) using negative binomials distribution for all models which had high overdispersion (Zuur et al. 2009). Site ID was used as a random factor to control the differences in sample size. I used a Java based MaxEnt standalone software version 3.4.1 (Steven et al. 2024) as a prediction model for smooth newts distribution.

ANOVA was used to analyze the relationship between water color and habitat types. I used linear model (LM) to test the relationship of water color and dissolved oxygen (DO%).

GLMM or LMM were used to examine the relationship between water color, habitat type and smooth newts a) body conditions, b) belly dot patterns and c) colorimetric variables. GLMM were used with negative binomial distribution due to strong overdispersion. Water color and Habitat types were used as independent (predictor) variable in models with smooth newt morphological variables as dependent variables. LMM was used in gaussian distribution to test the relationship between newts Fulton index and DO%. In all mixed models, site was used as a random factor. Statistical analyzes were conducted separately for each sex due to smooth newts having sexual dimorphism varying in size, color, and belly dot pattern characteristics. I investigated if chosen variables were depending on the date when the newt was captured. Date had low correlation with the selected variables, thus it was not used in the models.

I used the packages corrplot and GGally (Emerson et al. 2013) to investigate the Pearson correlations between body conditions, belly dot patterns and colorimetric variables. The variables were kept when they did not have strong correlation with each other. Appendix 1 presents the full list of correlations.

Weight had high positive correlation with SVL for male ( $r=0.842$ ) and female newts ( $r=0.912$ ). In addition, Fulton index had low correlation with SVL (males  $r = -0.059$ , females  $r = 0.035$ ) and weight (males  $r = 0.476$ , females  $r = 0.420$ ). Due to weight highly correlating with SVL, I decided to use the Fulton index in the subsequent analyses as a measure of body condition.

LMM were used when investigating the response of the Fulton index and tail length to water color and habitats whereas GLMM with negative binomial two distribution was used to assess the response of tail height to water color and habitats.

I conducted following newt analyses with following distributions:

I used gaussian distribution for the following colorimetric variables Tail\_B2\_UV, Tail\_H1\_UV, Tail\_S8\_Visual, Belly\_B2\_Visual, Belly\_S8\_Visual, Belly\_B2\_Total, Belly\_S4\_Total (Females in log), Belly\_S8\_Total, Tail\_B2\_Total, Tail\_S4\_Total, Tail\_S8\_Tota. Negative binomial two for Belly\_B2\_UV, Belly\_H1\_UV, Tail\_H2\_Visual, Tail\_B2\_Visual, Belly\_H2\_Visual, Belly\_H1\_Total, Belly\_H2\_Total, Tail\_H1\_Total and Tail\_H1\_Total.

I will only demonstrate the major important results in the results section, all LMMs and GLMMs results can be viewed from appendix 2.

To identify suitable habitat and possible impacts of forest practices in the landscape I used MaxEnt species ecological niche modeling. MaxEnt was chosen as a SDM because it only requires the information of species occurrence and also performs well even with low number of sample points. MaxEnt software was run using linear and quadratic features. Permanent water layer was removed due to high similarity in response with TWI. Spruce and Pine had very similar percentage of contribution, thus the coniferous data was used instead of them. AUC values were used to choose the resolution of the model. The MaxEnt model calculated from 50m resolution gave the highest AUC value of 0.912 followed by 100m (0.896), 300m (0.846) and 200m (0.851). I chose to use 50m and 100m models to compare the importance of resolution and to better understand how the landscape parameters affect the smooth newt's dispersal in EVO based on the MaxEnt models. Jackknife tests were used to evaluate the importance of environmental variables limiting the dispersal of smooth newts during breeding season.

### 3 Results

Out of the 42 sampled study sites 242 smooth newts (89 females and 153 males) were captured from 25 sites including 15 temporary wetlands, 10 permanent habitats (4 beaver sites and 6 lakes).

#### 3.1 Water quality

There was no difference in water color between habitat types (one-way ANOVA,  $F_{1,2} = 0.512$ ,  $P = 0.607$ ). DO% did not differ between habitat types (one-way ANOVA,  $F_{1,2} = 0.868$ ,  $P = 0.434$ , see figure 6).

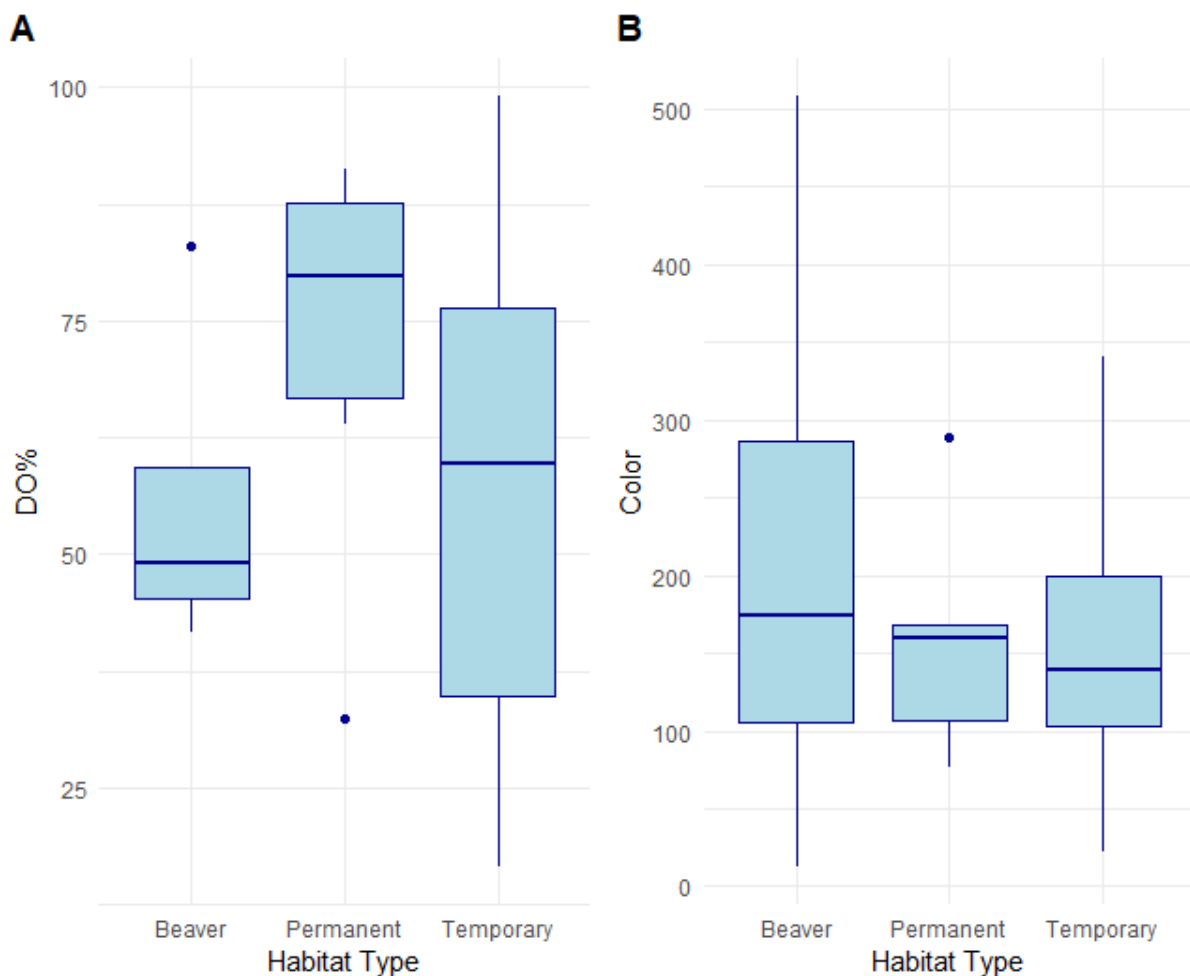


Figure 6. Differences between A) water color B) DO% in different three habitat types.

Dissolved oxygen (DO%) levels ranged from 16,6% to 99% and water color ranged between 13 and 508. No significant results were found between water color and DO% in all study sites (Linear model,  $DF_1 = 1$ ,  $DF_2 = 23$ ,  $\beta = -0.084$ ,  $P = 0.096$ ).



## 3.2 Smooth newts and brownification

### 3.2.1 Smooth newt body conditions

The Fulton index was negatively correlated to the water color for male newts (LMM, DF = 9.178,  $T = -2.521$ ,  $\beta = <0.0001$ ,  $P = <0.032$ , figure 7.1A) but not for females (LMM, DF = 19.72,  $T = -0.041$ ,  $\beta = <0.0001$ ,  $P = 0.968$ , figure 7.1B) only and no effect of habitat on either sexes.

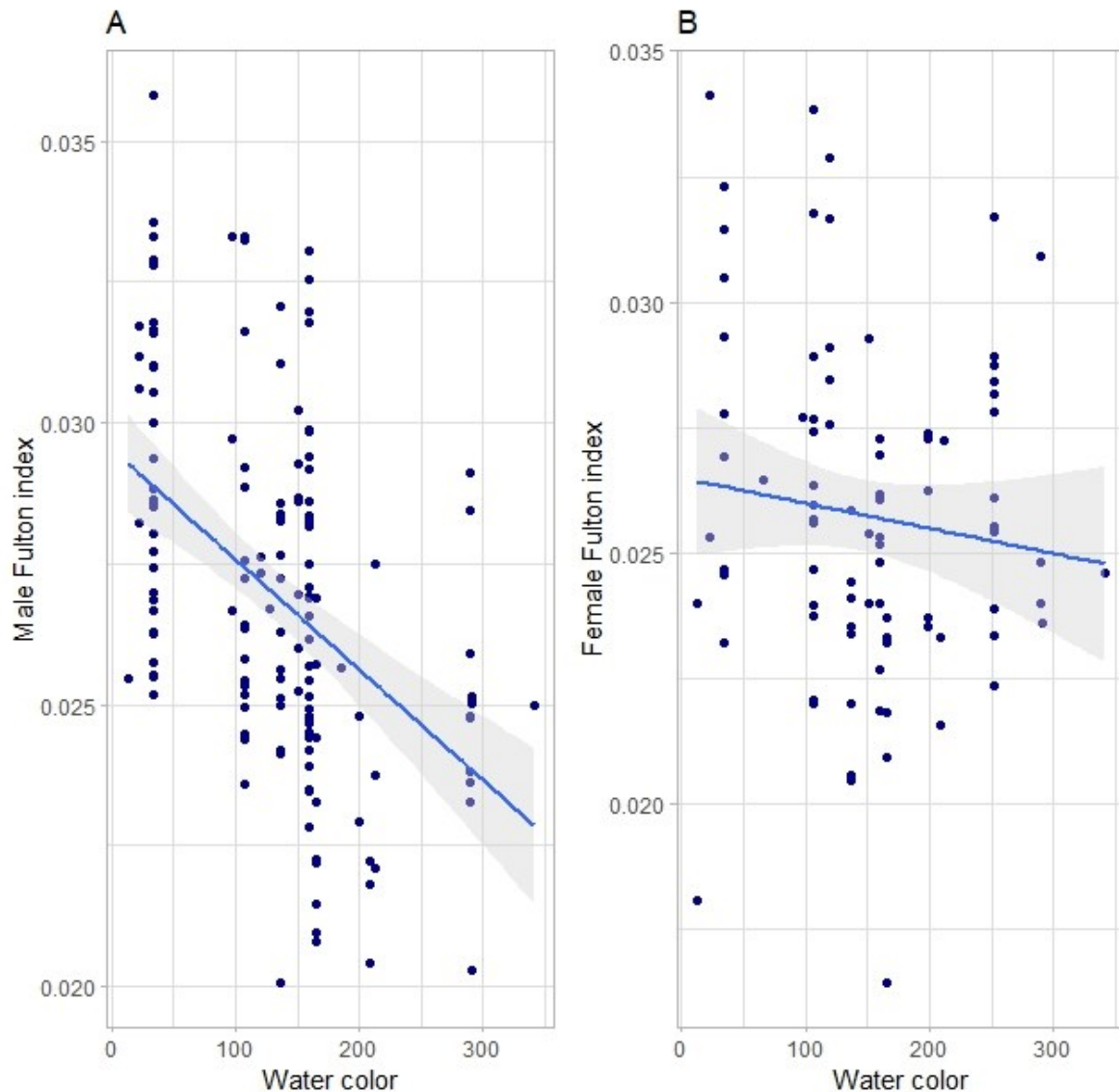


Figure 7.1. The relationship between smooth newts Fulton index for A) Male and B) Female newts and the water color.

Fulton index had no significant relationship with DO% for either male (LMM, DF = 11.33,  $T = 0.258$ ,  $\beta = 0.025$ ,  $P = 0.801$ ) or females newts (LMM, DF = 13.11,  $T = 0.310$ ,  $\beta = 0.024$ ,  $P = 0.762$ ).

### 3.2.2 Smooth newt belly dot patterns

Females had average count of 43,7 dots permanent habitats and 51.31 in temporary habitats. Males had average count of 21.71 dots in permanent habitats and 22.60 in temporary habitats. Smooth newts belly dot patterns did not respond to the water color (see appendix 1). However, the male newts did respond to the interaction of habitats and water color despite that there was no difference in the average number of belly dots between habitat types (see figure 7.2). Hence, male newts have significantly less belly dots when water color increases in temporary habitats but not in permanent wetlands (LMM, DF = 12.15, T = 3.197,  $\beta = 5.49$ , P = 0.008, figure 7.3).

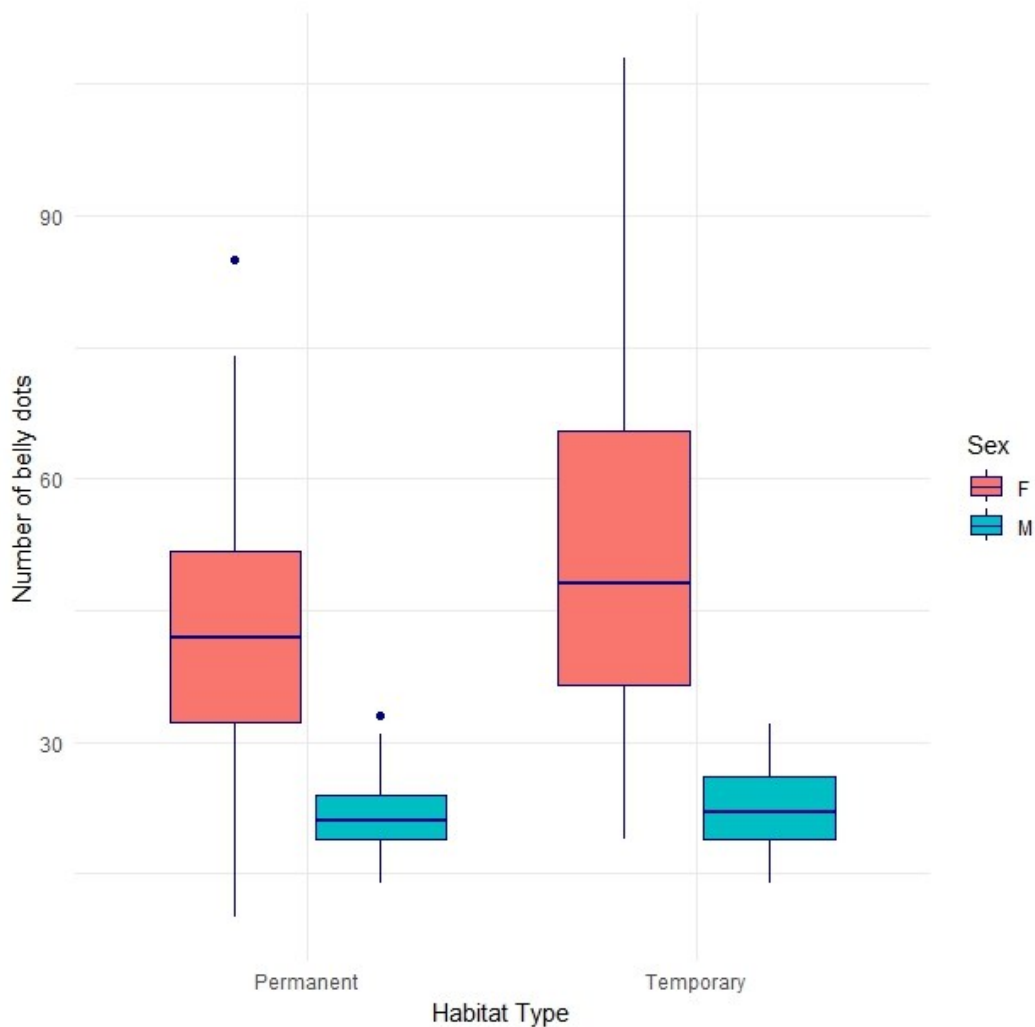


Figure 7.2. Average number of belly dots in each habitat per sex (Red = female and blue = males).

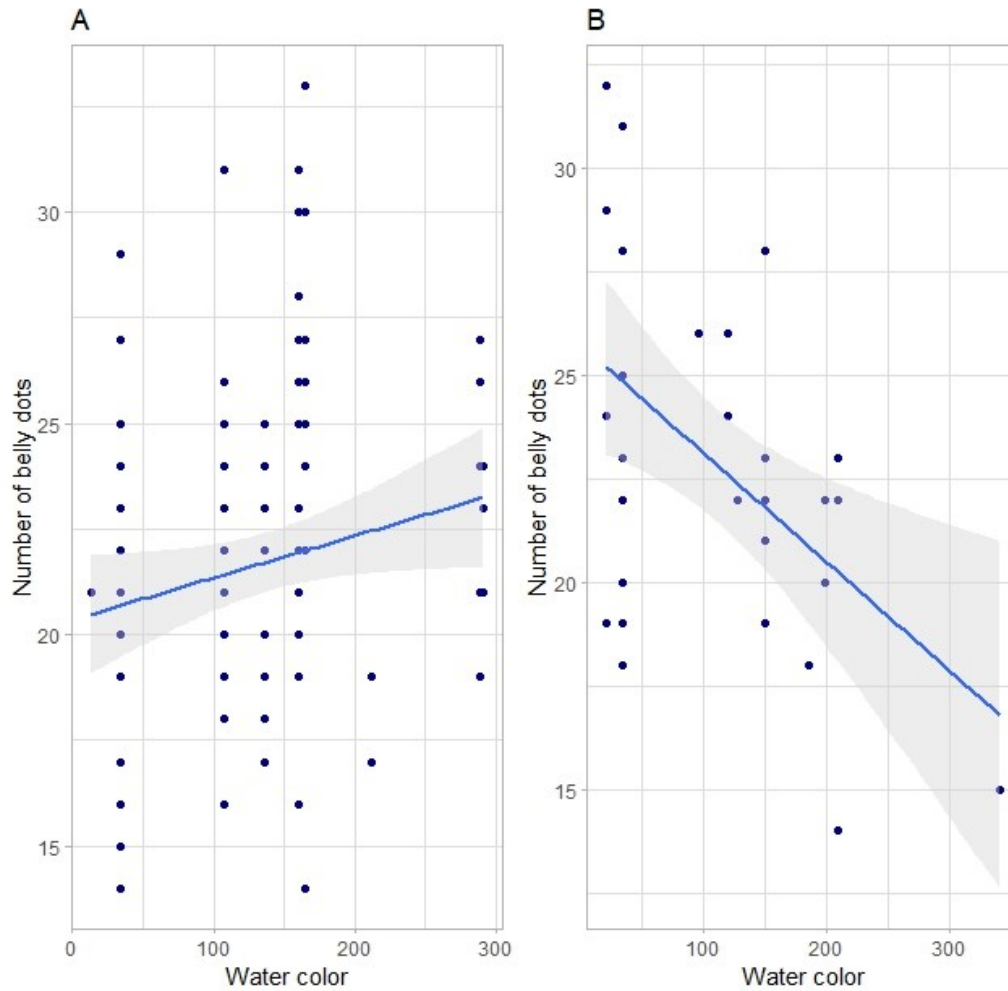


Figure 7.3. The relationship between water color and male smooth newts average belly dot count in sites of A) Permanent habitats B) Temporary habitats.

### 3.2.3 Smooth newt color response to water color

Male smooth newts tails hue peak wavelength (H1) in UV spectral range had positive relationship with water color in temporary but not in permanent habitats (LMM,  $DF = 9.12$ ,  $T = -9.12$ ,  $\beta = -16.79$ ,  $P = 0.034$ , figure 7.4).

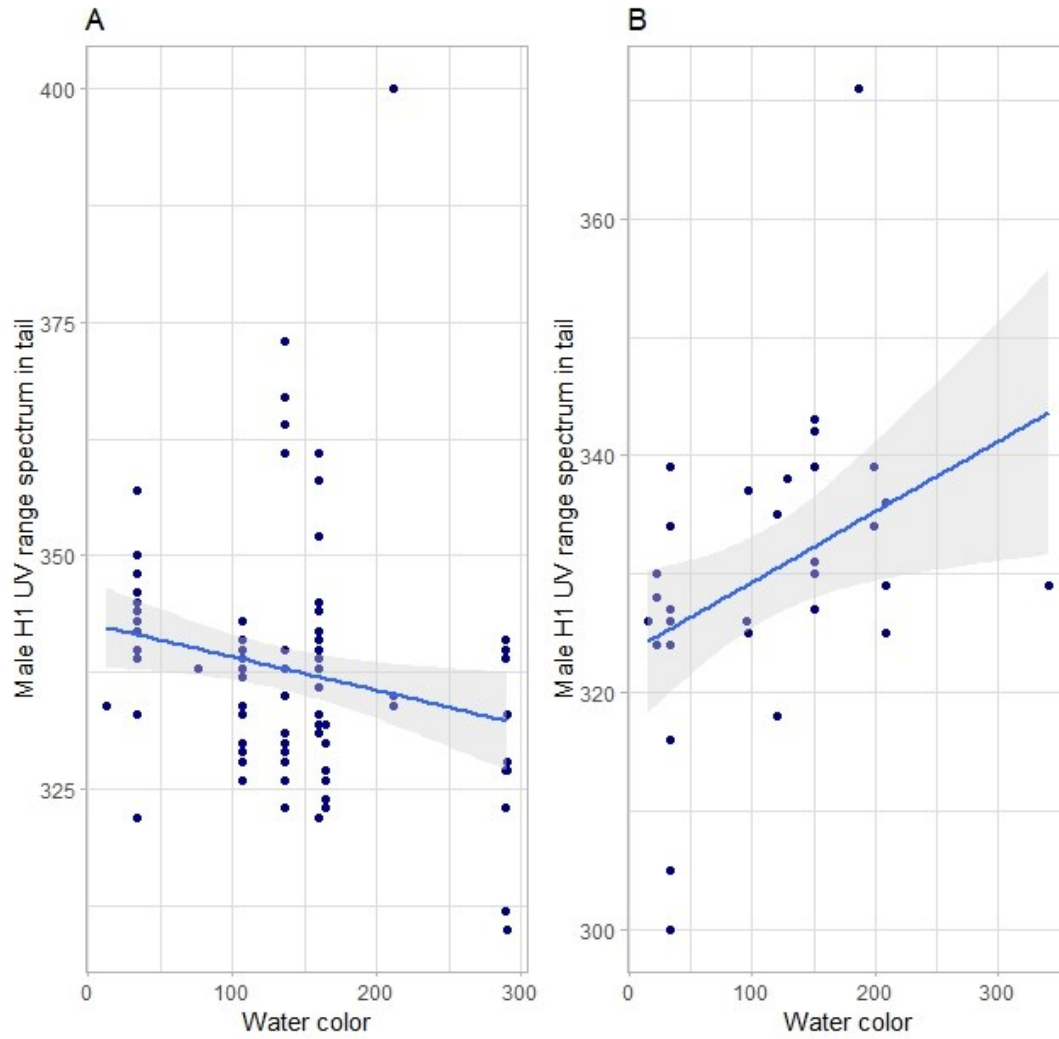


Figure 7.4. The relationship between male smooth newts' hue peak wavelength (H1) in UV spectral range in male newts tail and water color in A) permanent and B) temporary wetlands.

For females, hue in total spectral range (H2) had positive relationship with water color in temporary habitats but not in permanent ones (GLMM,  $N = 80$ ,  $Z = -1.962$   $\beta = -0.489$ ,  $P = 0.0498$ , Figure 7.5).

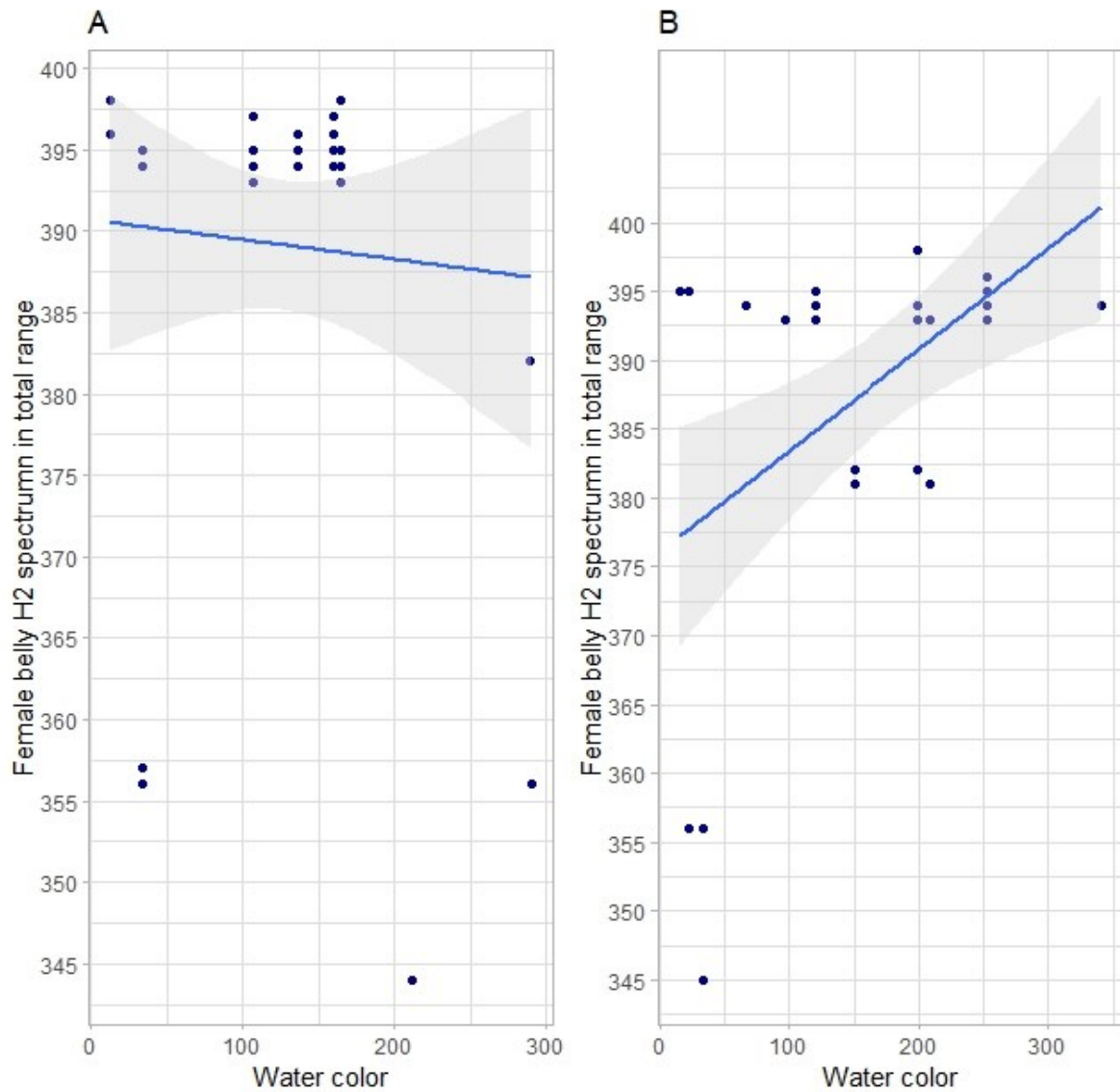


Figure 7.5. The relationship between female newts belly's hue in (H2) total range and water color in A) permanent and B) temporary wetlands.

Smooth newts tails hue peak wavelength (H1) in total range spectrum correlated positively with water color for males (GLMM,  $N = 136$ ,  $Z = 2.010$ ,  $\beta = <0.001$ ,  $P = 0.044$ , see Figure 7.6A) but not for females (GLMM,  $N = 80$ ,  $Z = 1.064$ ,  $\beta = <0.001$ ,  $P = 0.287$ , see Figure 7.6B).

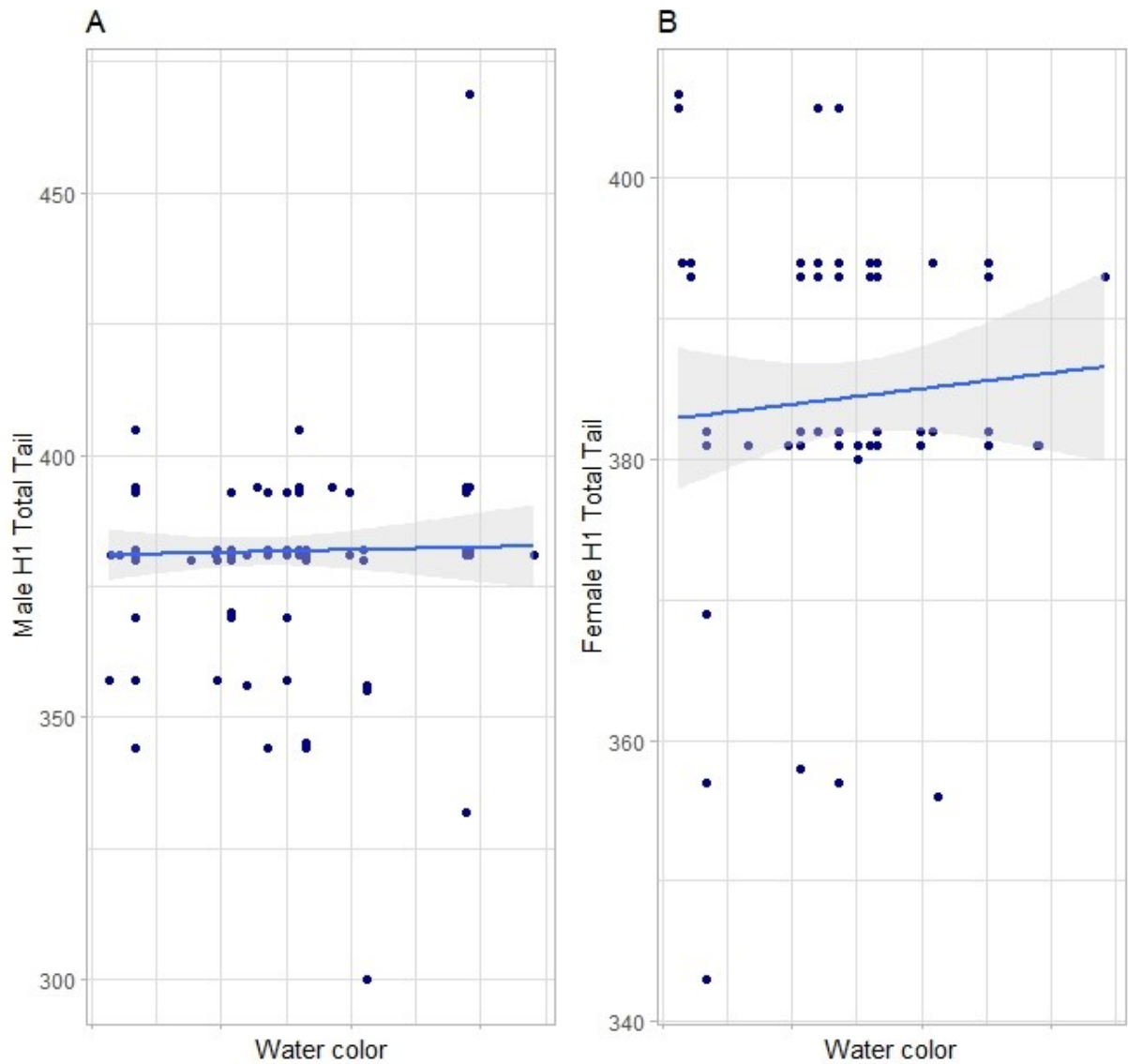


Figure 7.6. The relationship between smooth newts tails hue peak wavelength (H1) of total range spectrum for A) Male and B) Female newts.

### 3.3 MaxEnt models

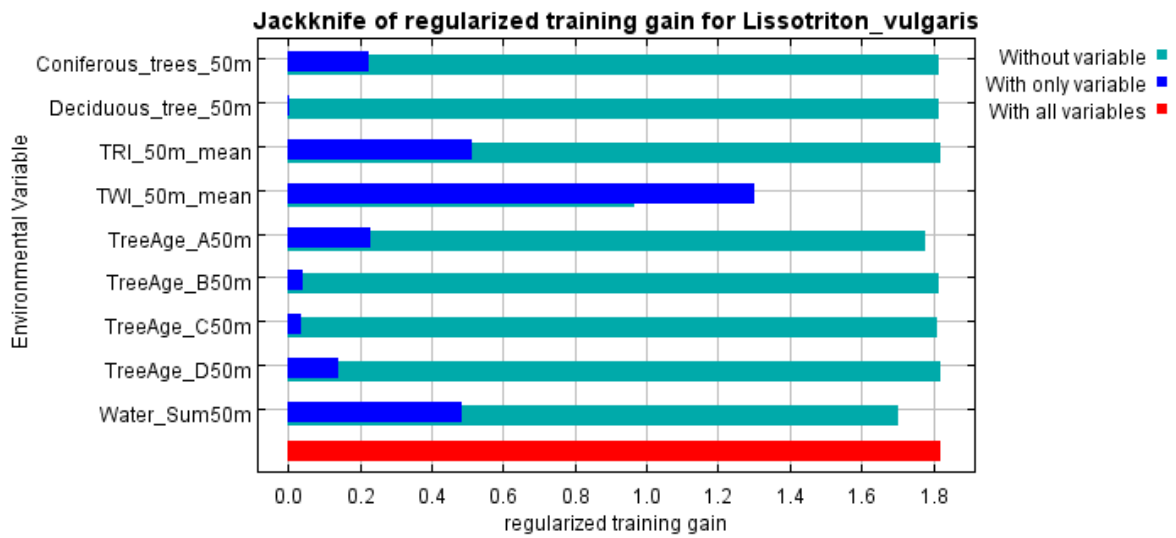
I used nine continuous environmental predictors in 50m and 100m resolution and 77 newt sites in the study landscape. Both models demonstrate that terrain wetness had the highest percent of contribution for smooth newts' dispersal during breeding season, but models show difference to second and third rank values (Table 2).

Table 2. Analysis of variable contributions in MaxEnt models of 50m and 100m resolution: The following table gives estimates of relative contributions of the environmental variables and smooth newt in the Maxent models. Variables in both models included terrain wetness index (TWI), terrain ruggedness index (TRI), deciduous and coniferous forests, tree age A (<2 years), B (2-10 years), C (10-20 years) and D (>20 years).

<b>Variable</b>	<b>Resolution</b>	<b>Percent contribution</b>	<b>Permutation importance</b>
<b>TWI</b>	<b>50m</b>	<b>66.2</b>	<b>84.6</b>
	<b>100m</b>	<b>33.7</b>	<b>36.2</b>
TRI	50m	0.2	0
	100m	3.9	3.9
<b>Tree Age A</b>	<b>50m</b>	<b>15.4</b>	<b>2.3</b>
	<b>100m</b>	<b>11.8</b>	<b>2.5</b>
Tree Age B	50m	4.1	1.7
	100m	5.6	2.8
Tree Age C	50m	0.6	2.7
	100m	2.3	2.8
Tree Age D	50m	0.0	0.2
	100m	3.7	3.2
<b>WaterSum</b>	<b>50m</b>	<b>12.5</b>	<b>4.6</b>
	<b>100m</b>	<b>27.3</b>	<b>9.3</b>
<b>Deciduous forest</b>	50m	0.5	0
	<b>100m</b>	<b>4.5</b>	<b>21</b>
<b>Coniferous forest</b>	50m	0.5	0
	<b>100m</b>	<b>7.2</b>	<b>18.2</b>

Jackknife results for the 50m model evaluated the most important single variable to be terrain wetness, followed by terrain ruggedness, coniferous forests, and the presence of water (water sum) (Figure 8 A). Whereas the 100m models most important single variable was also terrain wetness followed by water summary and other environmental factors having very low importance (Figure 8 B).

A)



B)

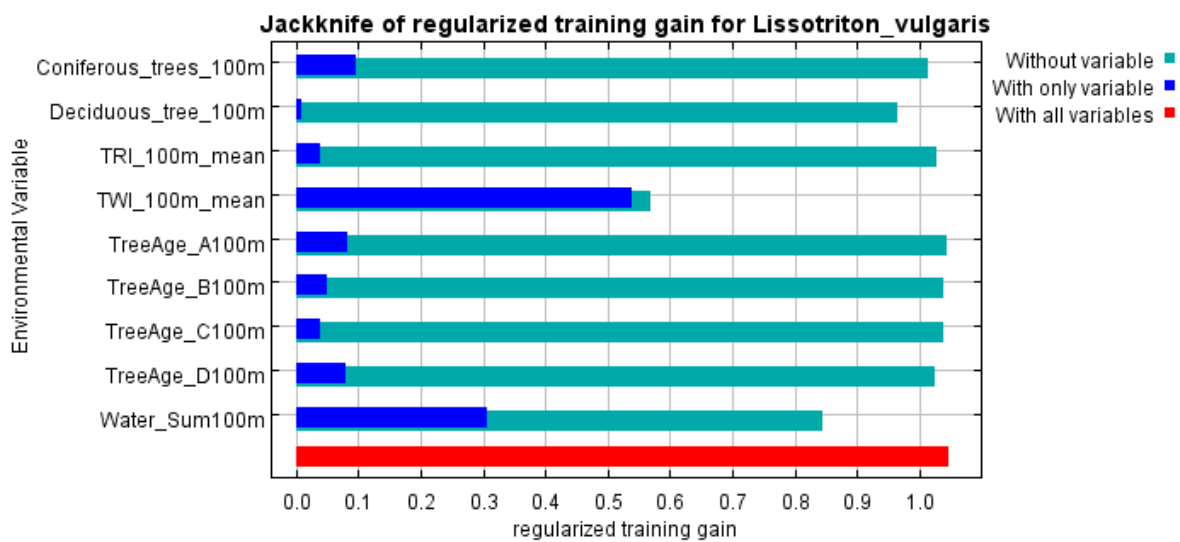


Figure 8. Importance of environmental variables to smooth newts during breeding season by jackknife analysis in A) 50m and B) 100m resolution models. Dark blue color represents the environmental variables with only using a single variable whereas the green color represents the without the single variable.



## 4 Discussion

This work studied smooth newts as a model species to understand the relationship between newts morphology and brownification in the context of water color changes. The results highlight smooth newts' morphological traits vary between sites; water color might influence male smooth newts body condition and the importance of temporary wetlands as a unique breeding habitat in a managed forest landscape. In addition, there were coloration and belly dot differences according to water color only in temporary wetlands but not at permanent wetlands. Hence, in landscape level habitat type and water color seem to be important factor contributing to smooth newts acclimation. Hence there is a possibility that this finding might be linked to adaptation. In future this could be tested with DNA analysis such as microsatellites or SNP analysis which could give us the answer if this is adaptation or not. In addition, the study demonstrated that smooth newts were found in varying forest habitats and water color, and no dispersal patterns were found related to forest management in the context of forest age. Nevertheless, the presence of smooth newt in EVO seemed to be mainly driven by the presence of water and landscapes humidity. Suggesting that the forest practices in EVO natura zone are strict enough not to have had significant influence on smooth newts dispersal at least according to my model. In the other hand smooth newts are widespread across Europe, which also indicate their ability to live in various environments and possibly that they are tolerant to human influenced landscapes. In general, amphibians which are less tolerant towards environmental change have shown to be influenced by forest activities such as timber harvesting (Patrick et al. 2006). Despite that I did not demonstrate that forest practices influence newts morphology in a forested landscape, other studies have found relationships between newts dispersal and human influenced landscape patterns. For example, degradation and loss of habitat was found to limit the dispersal of great crested newt (*Triturus cristatus*) (Nystrom et al. 2007). However, there are differences in newt species tolerance towards environmental change or their habitat choice. Smooth newts seem to be rather tolerant compared to many rare newt species toward environmental change. This was witnessed by Vuorio (2016) in study where sheltering vegetation was found to be more important for great crested newt compared to smooth newts. Thus, it is possible that the forest practices could influence other newt species morphological traits or even for smooth newts in a more managed forest landscape. Nevertheless, I could hypothesize that smooth newts are tolerant and are benefiting from metapopulation dynamics more than being restricted by the forest practices in EVO. Climate change is predicted to have increasing influence towards water

browning (Evans et al. 2006; Larsen et al. 2011), thus in future it is possible that the increasing water color may cause even more morphological differences between breeding sites.

During the summer 2023, compared to permanent wetlands, the studied temporary wetlands presented larger variation in water color and DO compared to permanent wetlands. Temporary wetlands lack fish which predate on newts, resulting to a habitat that is free from fish predation. Amphibians have shown responses to predation in their color (Rudh & Qvarnström 2013), behavior and morphology (Relyea 2001). For example, predation often decreases species foraging and mating activity time (Lima & Dill 1990). However, the relationship between DOC and amphibian foraging behavior was studied by Wagner (2016) but turned out to be insignificant whereas the predation risk was found to be significant. However, compared to Wagner's lab experiment study my thesis was concluded in natural environment. In addition, Wagner's study was carried out only for a short period of time, which in my opinion is a setup that has a high chance of getting significant results from predation. In addition, Wagner's (2016) study concluded with tadpoles which are different from newts. Although not being amphibians, previous study with fish larva in lab setup demonstrated that water browning did not have direct effect in their ability to detect prey but indirectly decreased their growth and survival due to decrease of micro-organisms (leech et al. 2021). The decrease of micro-organisms due to brownification was also observed by Wissel et al. (2003) in their study where they found that increasing water color increased abundance of *chaoborus* and switched the relative abundances of zooplankton towards larger species in lakes. Thus, I could hypothesize that directly or indirectly brownification could have the possibility to influence foraging behavior of newts in natural habitats. Lastly, I was not able to find much information between brownification and amphibians foraging abilities thus, there is a clear need for studies towards newts foraging behavior and brownification.

Despite not finding a statistically significant relationship between water color and DO there was a non-significant trend. Thus, it could be hypothesized that there is possibly a relationship between water color and DO%. Croijmans et al. (2021) found that oxygen was a better indicator of macroinvertebrates richness than temperature. Hence, DO could have possible impacts on the breeding sites community. In addition, the model of DO does not include the temperature which is an important factor when measuring DO due to the fact that water temperature correlates with DO% and is also influenced by time and depth (Antonopoulos & Gianniou 2003). Compared to permanent water bodies the temporary wetlands undergo more changes in their depth, temperature, and other environmental and

biological factors during the season, due to these factors the DO% measurements are correlating even more with the time of measurement compared to lakes. Despite the fact that this study could not demonstrate a significant relationship between newts body condition and DO% the previous findings state its importance for amphibians. Studies have shown that varying DO alter amphibians' behavior and development during their larva stage, Wassersug & Seibert (1975) showed that the effects of decreasing DO are species specific and stated that behavioral responses due to DO may be important in explaining the species distribution. Dmitrieva (2004) DO to influence the embryonic development of common toad (*Bufo bufo*) and Woods et al. (2010) claimed that low level DO is critical for the survival of salamanders. Newts' eggs are rather similar to other amphibian eggs and thus, could also be influenced by decreasing DO. Decreasing DO was also a concern of Saari et al. (2018) who demanded more research to understand reproductive threshold values of hypoxia to amphibians, invertebrates, and fishes. However, I did not measure larva development, but this could cause possible issues for newt populations in the future when climate change will increase water browning which has the potential to decrease DO. For newts Lukanov et al. (2021) found that buresch crested newt (*Triturus ivanbureschi*) are affected by oxygen levels in large ponds but not in small ponds. In addition, the decreasing amount of DO increases the change of hypoxia for newts and their prey. At the landscape scale water color is affected by landscape patterns which can be altered by human activities e.g. clear cutting. This is important due to the behavior of smooth newts returning every year to same breeding pond (Bell 1977) and their limited dispersal abilities (Kovar et al. 2009) the juveniles travel to close breeding ponds. Thus, we could hypothesize that if brownification decreases the amount of DO and disturbs development it would cause a decrease in juvenile fitness during early life and cause possible issues e.g. over wintering. In addition, brownification can have indirect effect to newt through food webs. Long term brownification has been observed to reduce the abundance of macroinvertebrates in lakes of EVO (Arzel et al. 2020), and low oxygen levels cause hypoxia therefore reducing benthic invertebrates especially non-mobile invertebrates (Karna 2003) which means less possible food for generalist predators such as smooth newts. Thus, concluding that brownification may reduce multiple important environmental factors which are beneficial for smooth newts during breeding season and early development. More research is needed to understand the cumulative impacts of brownifications in breeding habitats. In addition, I suggest studies related to brownification would look to the possible relationship between water color and DO and, the impacts of DO to newt populations especially during their early stages of development in varying environmental conditions including habitats size.

Water browning influences the visual environment of many amphibian species during their aquatic phase. Thus, water color possibly influences individuals traits which are driven by visual clues e.g. predation risk or mating behavior. For example, smooth newts use visual clues during their mating behavior (Secondi & Théry 2014). Previous studies have demonstrated that newts sexual dimorphic characteristics can be driven by environmental conditions. Secondi et al. (2007) found that palmate newts (*Lissotriton helveticus*) sexual morphology was affected by water turbidity. Thus, it is possible that other water properties such as color could drive similar changes to smooth newts' sexual morphology. Hence, I predicted that smooth newts body conditions would respond to visual change caused by the increasing water color especially, in traits which are part of sexual dimorphism. Despite tails importance in the breeding patterns of smooth newts, I did not find any significant results between water color and tail morphology. However, I found that the increase in water color had negative correlation with males Fulton index. Meaning that in this one-year study brownification in the context of water color had negative relationship with male smooth newts body condition but not with females. In long-term in the context of brownification this could lead towards the decreasing body condition of male newts. In this work the body condition measure included the male's secondary sexual trait, their dorsal crest and could explain why males had significant response but females did not. This could be due to resource allocation towards sexual dimorphic traits. Resource allocation is expected to drive sex specific differences between individuals especially when species display sexual dimorphism (Ram 1984; Frank 1987; Wild et al. 2007). Angelini et al. (2015) found that higher temperature drives the southern populations of Sardinian mountain newt (*Euproctus platycephalus*) to reach maturity faster compared to more northern populations. These studies show that newts sexual dimorphic traits can be driven by both biotic and abiotic factors. Thus, the result could be driven by sex specific resource allocation where male newts may have invested less resources towards displaying their dorsal crest during the breeding season when water color increases. De Lisle and Rowe (2014) found that red-spotted newts altered their resource allocation on secondary sexual traits due to competition and social environment. Thus, newts have shown both sex specific and non sex specific response and it is possible that brownification also has sex specific responses as witnessed in this study.

Environmental selection can influence evolution of both non-sexual and sexual traits of many species (Andersson 1982), and environmental variables correlate with resource allocation of sexual traits (Macías-Ordóñez et al. 2013). This could mean that water color has the possibility to influence newts color. Previous studies have demonstrated that males UV

range hue to be attractive colorimetric variable for the female newts while choosing their mating partner (Secondi et al. 2012). Thus, I predicted the smooth newts to display higher or duller mean or maximum colorimetric in darker waters. I did not find correlation between UV spectral range colorimetric variables and water color when habitat type was not included. This could possibly be that in boreal forests UV clues are not that important for smooth newts and possibly use other clues for sexual selection. However, responses between different habitat types supported my prediction. In addition this prediction is supported by the risk allocation hypothesis (Lima & Bednekoff 1999). Here, in this study smooth newts demonstrating colorimetric responses to water color only in temporary wetlands but not in permanent waters. Zapletalová et al. (2016) found that moth species which prefer hygrophilous habitats display higher UV reflectance from their wings compared to species preferring different habitat types. Bohórquez-Alonso & Molina-Borja (2014) claimed that the habitat differences in Southern and Northern Tenerife have caused the current differences in UV-blue reflectance between two subspecies of Tenerife lizard (*Gallotia galloti*). I found an increase in male newt tails hue peak wavelength in UV spectral range in temporary wetlands when water color increase but not in permanent waters. This could be possibly due to competition or as a response to water color resulting to male newts allocating more resources to sexual traits. I could hypothesize that it could be that in context of water color the female newts visual clues to detect males brightness from color becomes more limited when water color is darker, thus allocating more resources towards the brightness would be beneficial for the male newts. In long-term brownification could possibly cause smooth newts to have subspecies due to habitat differences as claimed in the lizard study by Bohórquez-Alonso & Molina-Borja (2014). Nevertheless, in such fine scale of landscape as evo for subspecies to form there would have to be very high selective force toward water color and habitat type. Another aspect here is that in temporary wetlands the lack predation could possibly result in newts having more time to forage. Hence the possibly in long term the absence of predators and higher abundance of invertebrates being beneficial environmental factors of temporary wetlands for male smooth newts when habitat quality decreases in the context of water color. It could be that in the small fishless temporary wetland's newts invest more towards sexual traits, therefore showing phenotypic plasticity towards the water color. This is supported by the previous finding that skin color brightness increases amphibians predation risk (Tattersall et al. 2006). In addition to UV spectrum, I found that the male smooth newts tails hue peak wavelength (H1) in total range spectrum had positive correlation with water color for males. For females' the belly hue in total spectral had positive correlation with increasing water. It is likely that mixed selective

pressures are driving colorimetric variables for species which have sexual signals (Wolfenbarger 1999). Carlson and Langkilde (2016) suggested that morphological traits of Red-Spotted Newt (*Notophthalmus viridescens viridescens*) have either phenotypic plasticity or local adaptation as a response to food availability. This finding supports my prediction that newts morphological traits could be changing due to environmental conditions. Thus, it could be that the newts are able to adapt in temporary wetland but not in permanents due to availability of resources. Nevertheless, the newts Fulton index did not have any different responses between habitat types, thus the colorimetric findings should not correlate with the smooth newts age or early development. Therefore, the findings of colorimetric sexual traits suggest that these are phenotypic plasticity responses to an increase of water color when more resources are available to be spent.

Unlike colorimetric variables the belly dot patterns of smooth newts do not change during their lifetime, they are not plastic and are more likely that the patterns are driven by long term evolution (Mettouris et al. 2016). It is not well understood what the role of the dark belly dots are for smooth newts. In the landscape of EVO either male or female smooth newts belly dot patterns did not respond to water color. Suggesting that water color is not driving change in the belly dot patterns. However, I found that smooth newt males have less belly dots in temporary wetlands when water color increase but not in permanent waters. Arntzen et al. (1993) found that newts get more belly dots and the dots grow in size as they age. Mettouris et al. (2016) found that smooth newts belly dots do not grow much during adulthood. Concluding these two findings, the age-belly dot relationship is likely gradual increasing in size and number when newt matures, but the changes are minimal when reaching adulthood (Arntzen et al 1993; Mettouris 2016). Hence, when looking from the aspect of this thesis where all of the newts were already adults, having made their way back to the breeding sites. Thus, it would be hard to explain the finding by male newt's age. I can hypothesize that even if age does contribute towards the finding it is unlikely the only reason behind the finding. Smooth newts have four species which are hard to tell apart without molecular methods. These four species distributions have low overlap (Skorinov et al. 2022). However, recently Skorinov et al (2022) found that three of these species' males, including smooth newt, kosswig's smooth newt (*Lissotriton kosswigi*) and schmidtler's smooth newt (*Lissotriton schmidtleri*) can be identified from their dark belly dots. This suggest that smooth newt males have very similar belly dot patterns with each other. However, they did measure different patterns compared to what was done in this thesis and between species for different purpose. De Solan et al. (2021) found that *lissotriton* newts belly patterns are likely reflecting their

adaptation to local environment. Hence, there is growing information of newts adaptation to environment (Secondi et al. 2007; De Solan et al. 2021; Skorinov et al. 2022). Thus, it could be possible that the finding is a response to habitat types such as temporary wetlands. Regarding the finding I admit that the results trend is mainly driven by 2 sites (outliers) and caution should be applied when interpreting the result. However, if we consider this finding reliable, I could hypothesize that this could be due to plasticity. I could hypothesize that the number of belly dots decrease the individuals change of being detected by a predator in darker waters and that females prefer males with more dots. In fishless temporary wetland there is less aquatic vertebrate predation, and more belly dots are not beneficial for the newts to avoid predation, therefore the selective pressure between habitats changes due to survival and breeding rates. This could possibly cause the differences between the habitats; however, I admit that further research is needed to fully understand the reasons behind this.

Landscape characteristics influence amphibian populations (Hermann et al 2005). In this study using MaxEnt modeling forest age did not seem to visibly contribute to smooth newts dispersal. Forest age had very low correlation with study sites water color (see appendix 3). Thus, the findings in this thesis were against the findings of DeMaynadier et al. (1995), where they found clear cutting to be important for amphibians' and especially to salamanders. Whereas Vuorio et al. (2015) in their study came to a similar conclusion as this study that smooth newts had low influence from forest age. Nevertheless, forest practices influence amphibians are highly depending on the species (Smith et al. 2018). In addition, forest practices tend to have different results depending on the scale of the study (Harper et al 2015). It could be that the smooth newts do have local extinction due to forest practices which are not witnessed when studying landscape level where the extinctions are less common (Harper et al 2015). The issue with Forest practices is its impact causing degradation of habitat quality and extent contributing to amphibian decline in Forested landscape (Harper et al. 2015) such as clear cutting increase the temperature and decrease the humidity at the ground level, potentially impairing the dispersal capacities of newts. However, in EVO the forest practices are limited, it could be possible that in a more managed landscape the smooth newt's dispersal would be limited. In addition, not all forest practices have the same impact. To compare the forest practices for example, EM practices use clear cutting methods typically followed by soil preparation and tree planting or seeding. This is done solely for timber production purposes, supporting homogenous environment and intensive land management where natural succession of forest is minimalized. Ditch network maintenance (DMN) is used to promote forest growth at peatlands by increasing humidity and water level, but it also increases

nutrient and sediment load having negative effect to water quality (Miettinen et al. 2020). Joensuu et al. (2002) also stated that DMN is considered to be harmful forest management method due to increased export of the sediment. Nieminen et al. (2018b) suggest using CCF instead of EM due to potential increase of overall income at drained peatlands. This income increase would be due to less need of forest maintenance practices that are often recommended for every 20 years to sustain drainage conditions (Härkönen et al. 2023). DMN is less needed because of the change in variation of evapotranspiration and peatlands are less likely to dry. Thus, the forest practices do not only contribute to the forest age but also to the habitat quality. Thus, I suggest that while modeling SDMs for forest practices more habitat quality variables should be used. This would possibly reveal trends which have not been demonstrated in this study. I would say it is notable that SDMs should be conducted in multiple resolutions. For example, in this study I concluded four models of SDMs in different resolutions. The models demonstrated that the forest age in any category had minimal importance in both 50m and 100m resolution models. In addition, the MaxEnt finding of terrain wetness is consistent with Rohr & Madison (2003), which demonstrated the importance of humidity. However, comparing the two models revealed that in 100m TRI loses its importance compared to the 50m resolution model. It could be that in higher resolution the water summary and TWI is overvalued for their importance in EVO and other environmental variables are suffering from too high resolution. In the 50m resolution model jackknife TRI's importance was as important as the water summary, indicating that newts could possibly avoid or are unable to disperse to direction which have high elevation difference. Similar findings of elevations importance were concluded by Naumov et al. (2020) using MaxEnt found that elevation was the best predictor indicating dispersal of Alpine Newt (*Ichthyosaura alpestris*) in Bulgaria. However, their study is much concluded in much broader scale compared to my MaxEnt model. Their elevation and slope resolution were 40m which is very close to my 50m resolution. In addition, Hernandez et al. (2017) found that crocodile newts (genus *Echinotriton*) distribute areas from low to moderate elevation, thus newts avoiding or being unable to pass high elevation. Haugen et al. (2023) found that increasing human impact causes the decrease genetic diversity and gene flow for great crested newts across Norway. They also found that at landscape level the gene flow is highly influenced by moisture, vegetation cover, and land cover conversion for the pond-breeding newt. Antunes et al. (2023) stated that smooth newts are generally found in low elevations, woody habitats and due to their large area of distribution they show high tolerance and low sensitivity to human influence. Antunes et al. (2023) argued smooth newt to be ecological



generalist. To conclude the dispersal modeling I suggest that smooth newts have high tolerance at least towards to limited forest practices in a managed forest landscape as long as their breeding ponds are connected to terrestrial and the distance for juveniles are not too far. In the landscape of EVO there are plenty of lakes and temporary wetlands, thus finding a breeding habitat should not be an issue for juveniles looking out for mates. In addition, most parts of the EVO landscape have very high humidity and continuous forest coverage which are beneficial for newts. I would suggest that the effects of forest practices be studied with another species than smooth newts due to their seemingly high tolerance.

However, the model is suitable for evaluating forest practices in context of forest age but mostly ignores the habitat quality. For example, MaxEnt model used in this study mostly ignores important biodiversity features, for example the breeding site quality (e.g. water color, food sources) and to some extent human impact (e.g. agriculture, road networks). Forest age is not the only environmental feature changing due to clear cutting, forest practices cause other direct and indirect effects. Popescu & Hunter (2011) found that clear cutting alters important environmental features of smooth newts by lower terrain wetness and destroy important temporary wetlands possibly disturbing the connectivity between their terrestrial and aquatic habitats. Wetland connectivity is especially important for species like smooth newts which have poor, short dispersal abilities (Buono et al. 2023). However, in EVO the forest practices do not seem to disconnect the connectivity between habitat types. Newts are very commonly found across the landscape, thus suggesting that connectivity of wetlands does not seem to be disturbed by forest practices in Evo. In addition, in the MaxEnt models jackknife analyze none of the forest ages was given high importance as environmental variable. The MaxEnt model supports previous findings from connectivity studies concluded in Sweden where they found that protected landscapes have good genetic flow whereas in urban landscapes there are more variation (Davis 2020). This finding is against the prediction that forest practices would affect smooth newts' dispersal. In addition, I used DO% and color as measures of habitat quality. In addition, there was a low correlation between mean forest age and color or DO% (see appendix 3). Thus, it is very unlikely that in context of the chosen variables the forest practices in EVO are contributing towards water color or DO% in the context of forest age in the study landscape. It is to be noted that using other variables could reveal something that my model did not demonstrate. According to results of the SDM the forest practices which are often limited in natura 2000 zones are not having effect to the body conditions of smooth newt in the context of water color or forest practices. However, to have a wider understanding of landscapes effect to the population one should include analyses of

landscape genetics which could possibly reveal genetic differences between sites and landscape variables.

Here I have discussed the responses of smooth newts to water color and the importance of temporary wetlands for smooth newts. In landscape level smooth newts are responding to water color in context of morphological variables and also their breeding site quality is highly important. Similar trends of connectivity, presence of fish and land use were identified to impact smooth newts' occurrence and abundance in urban areas (Vehkaoja et al. 2023). I suggest that further studies are needed to understand how brownification affects amphibians' behavior and survival, especially during their early development.

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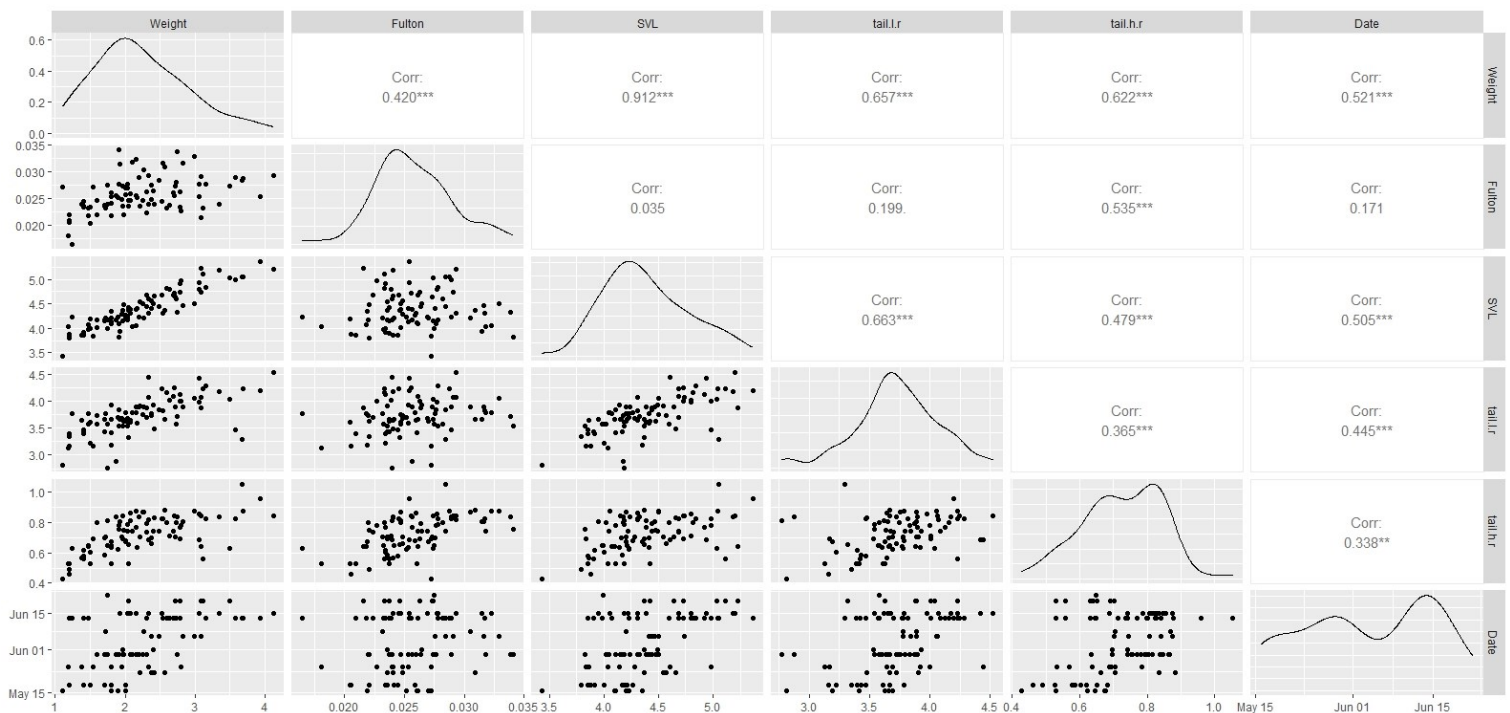
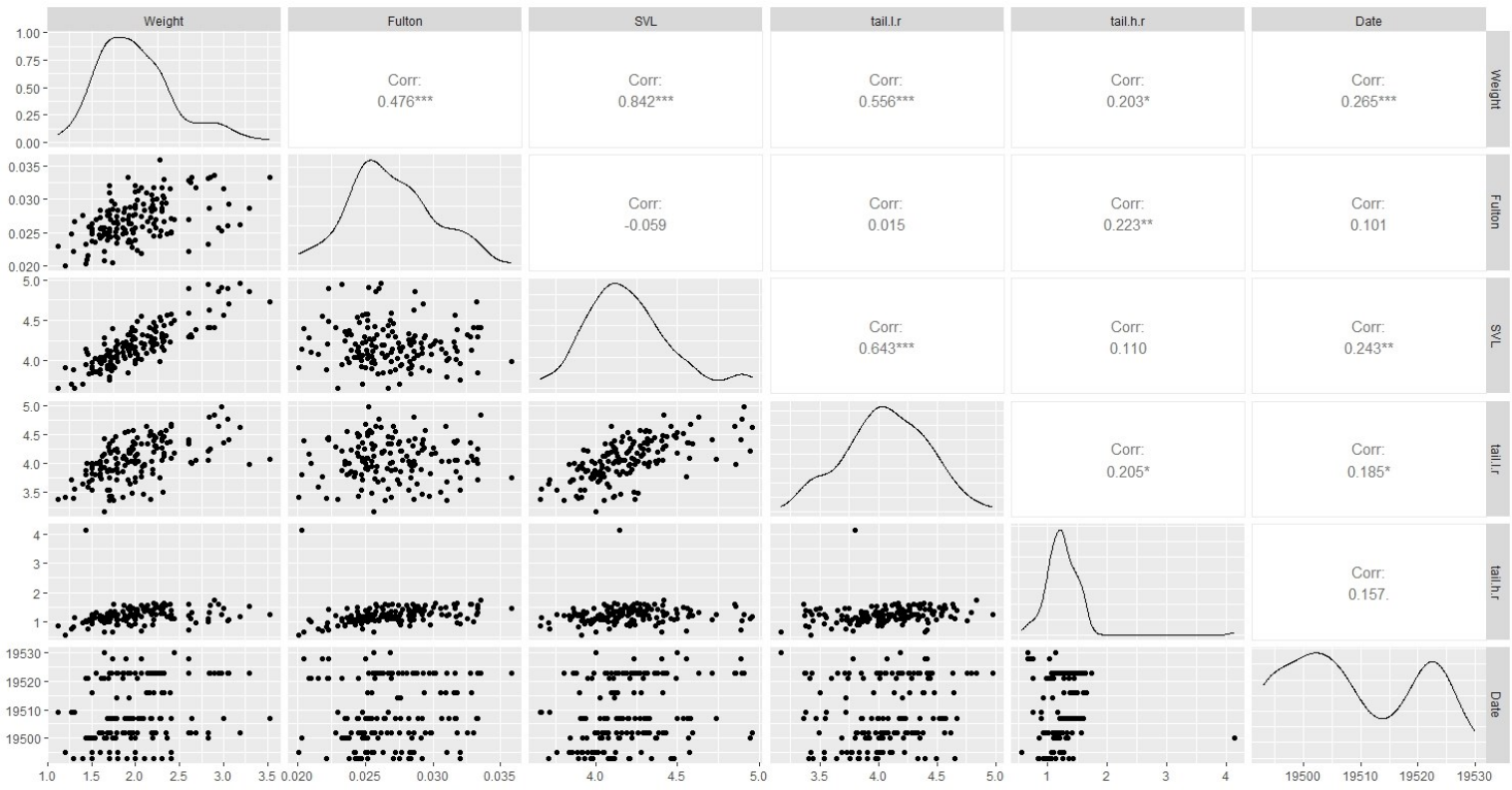
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## 7 Appendices

Appendix 1. Correlation matrix of A) male and B) female newt body conditions, belly dot patterns and colorimetric variables.

Appendix 1.1 A) Male newts body condition correlations.

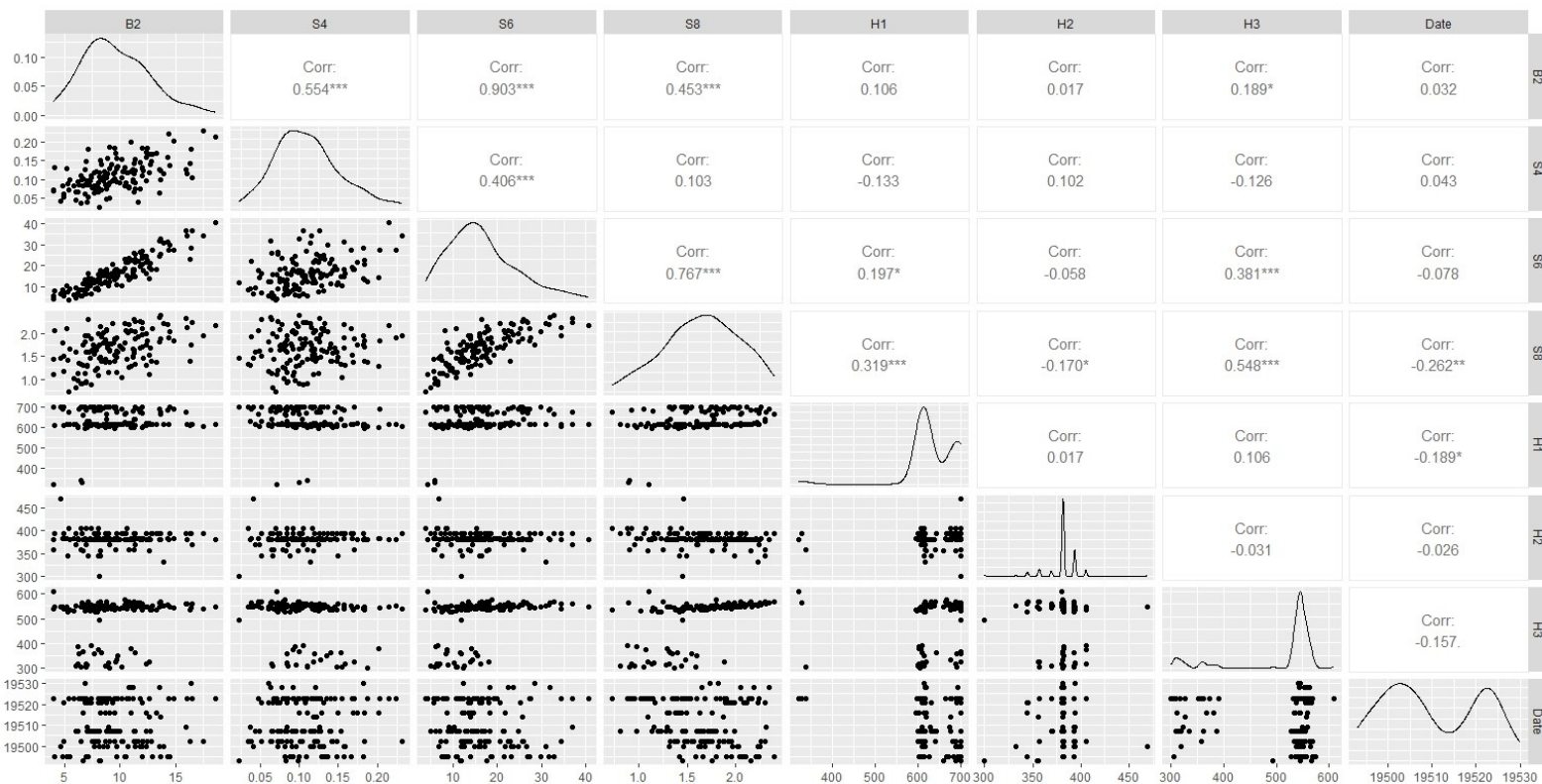


Appendix 1.1 B) Female newts body condition correlations.

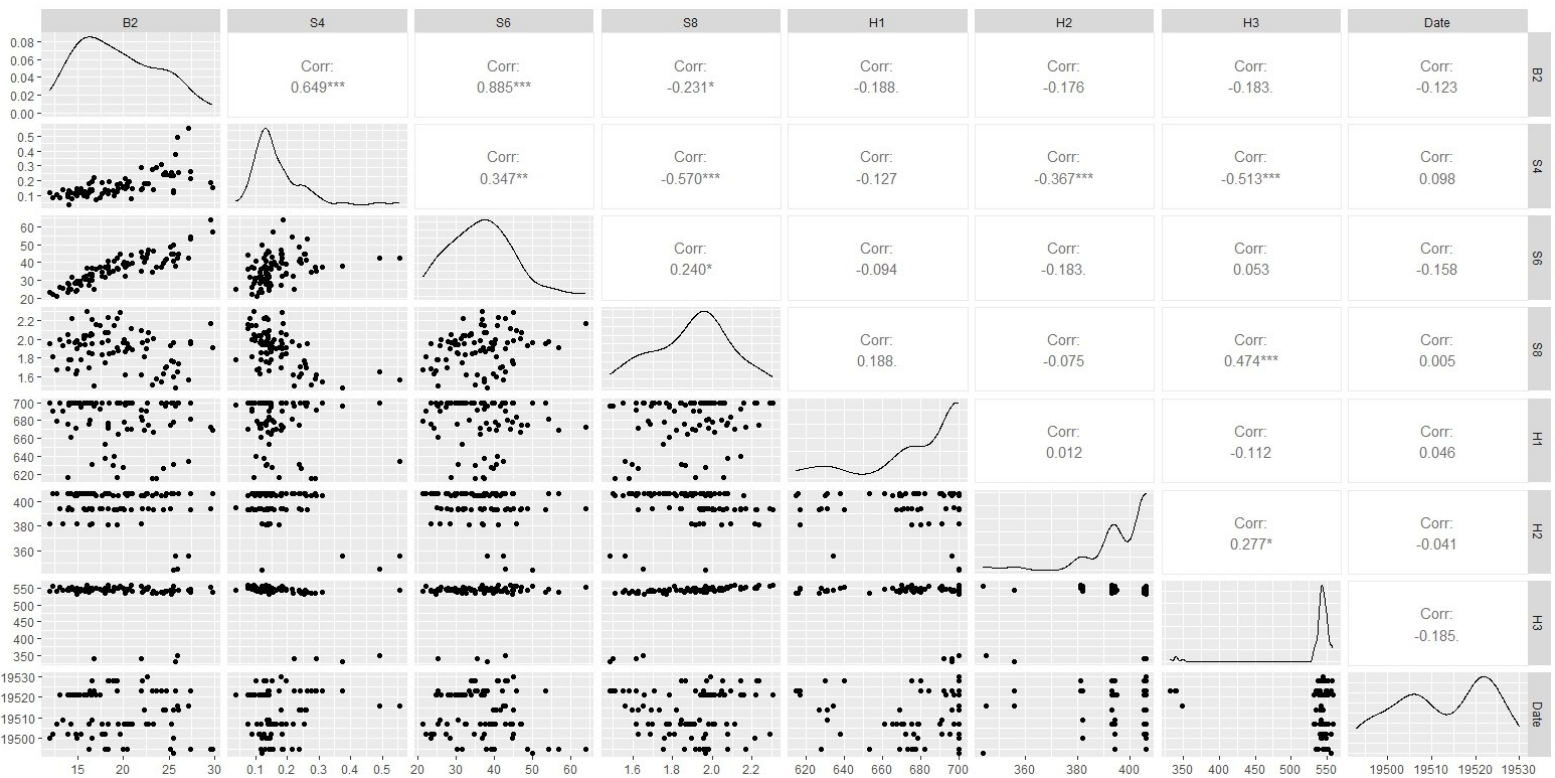




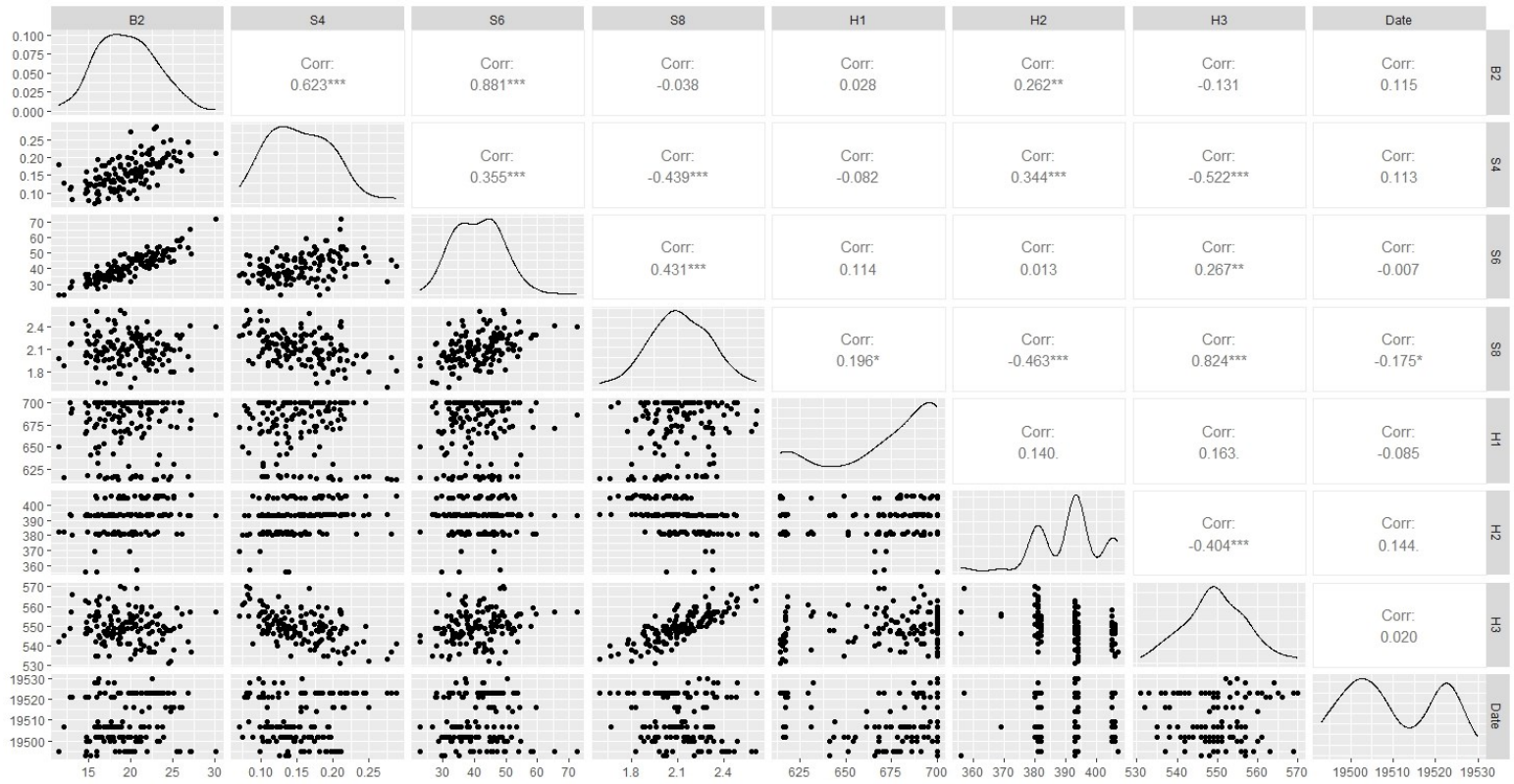




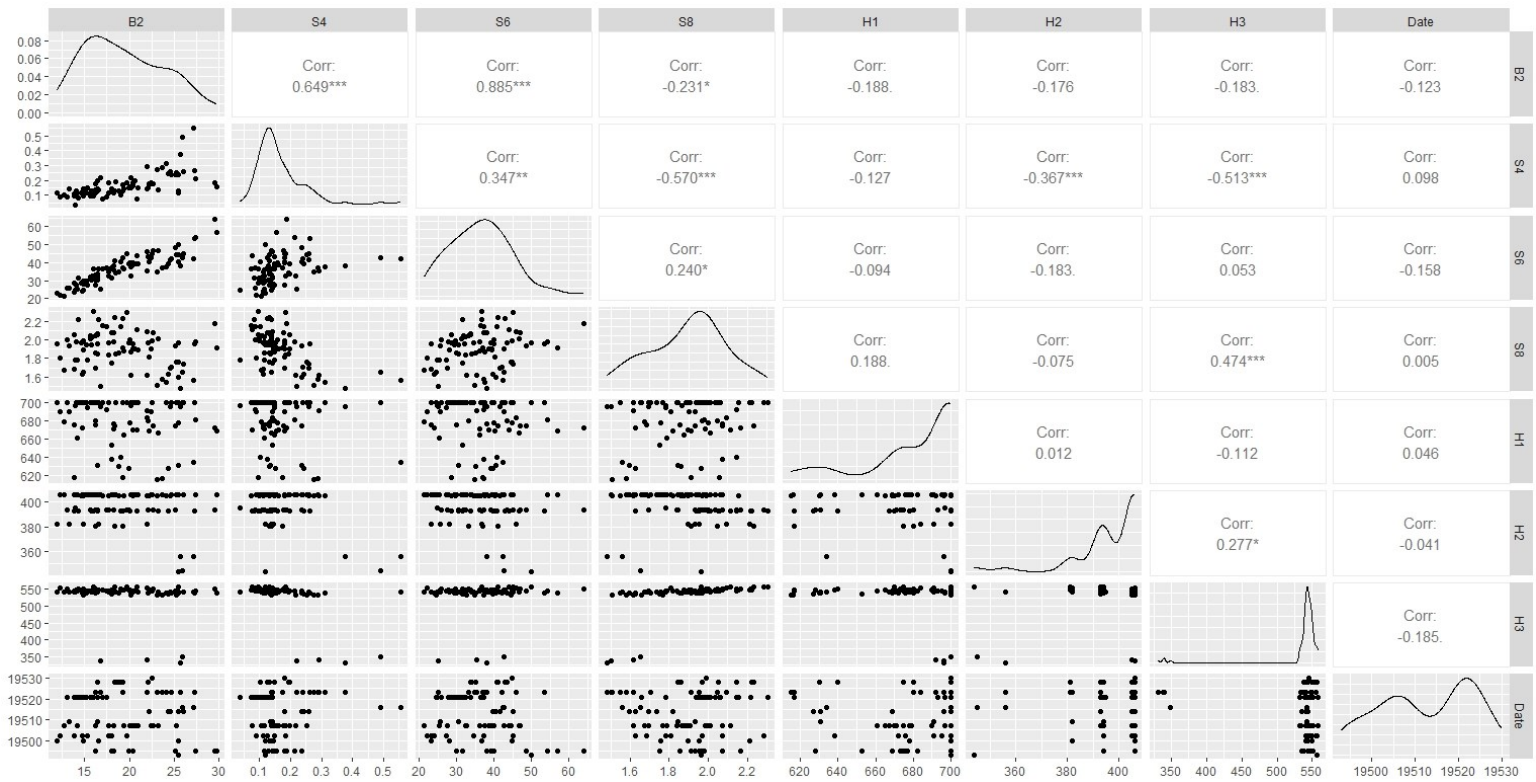
Appendix 1.31 A) Male newts tail total spectrum correlations.



Appendix 1.31 B) Female newts tail total spectrum correlations.

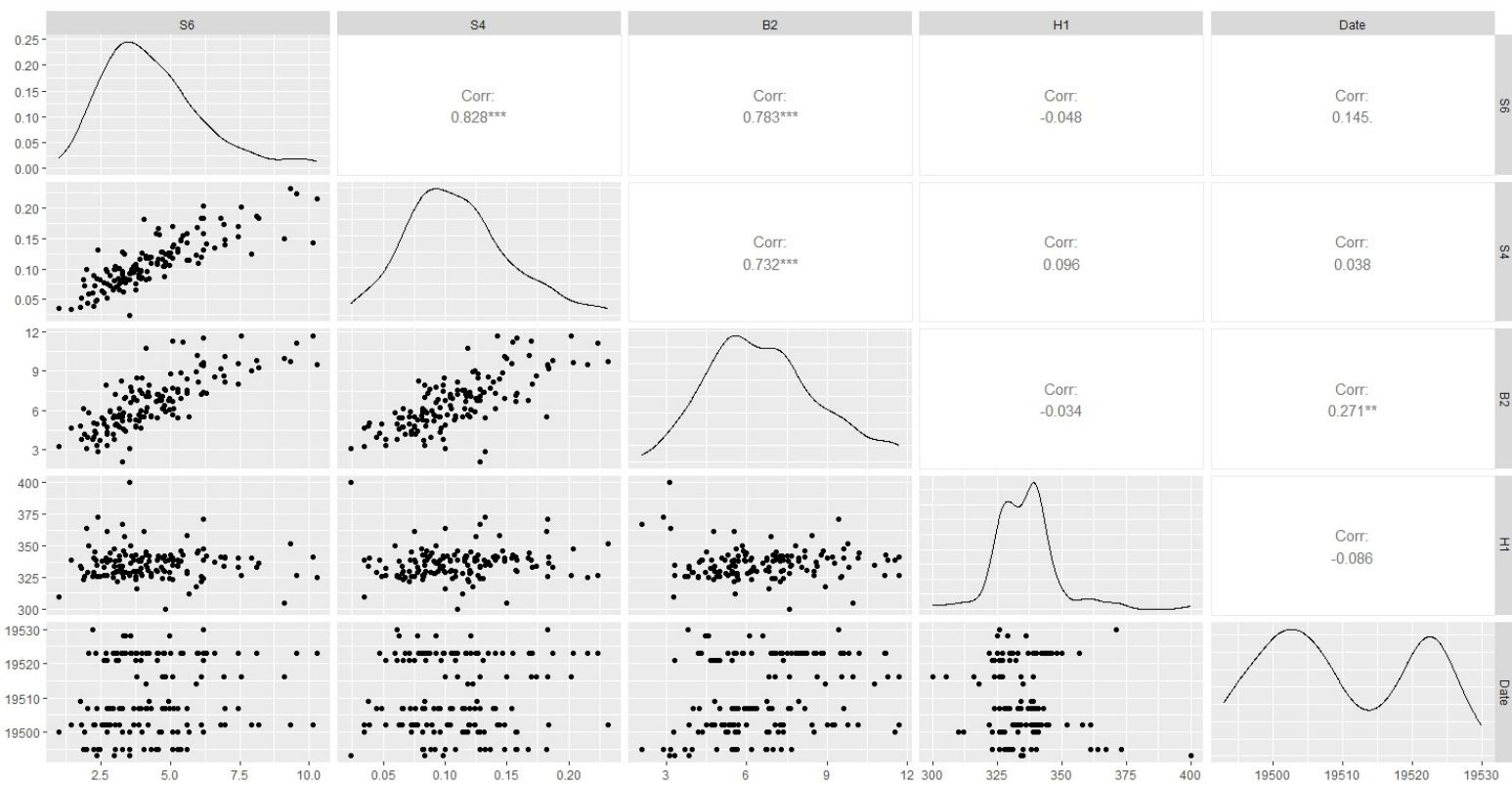


Appendix 1.31 A) Male newts belly total spectrum correlations.

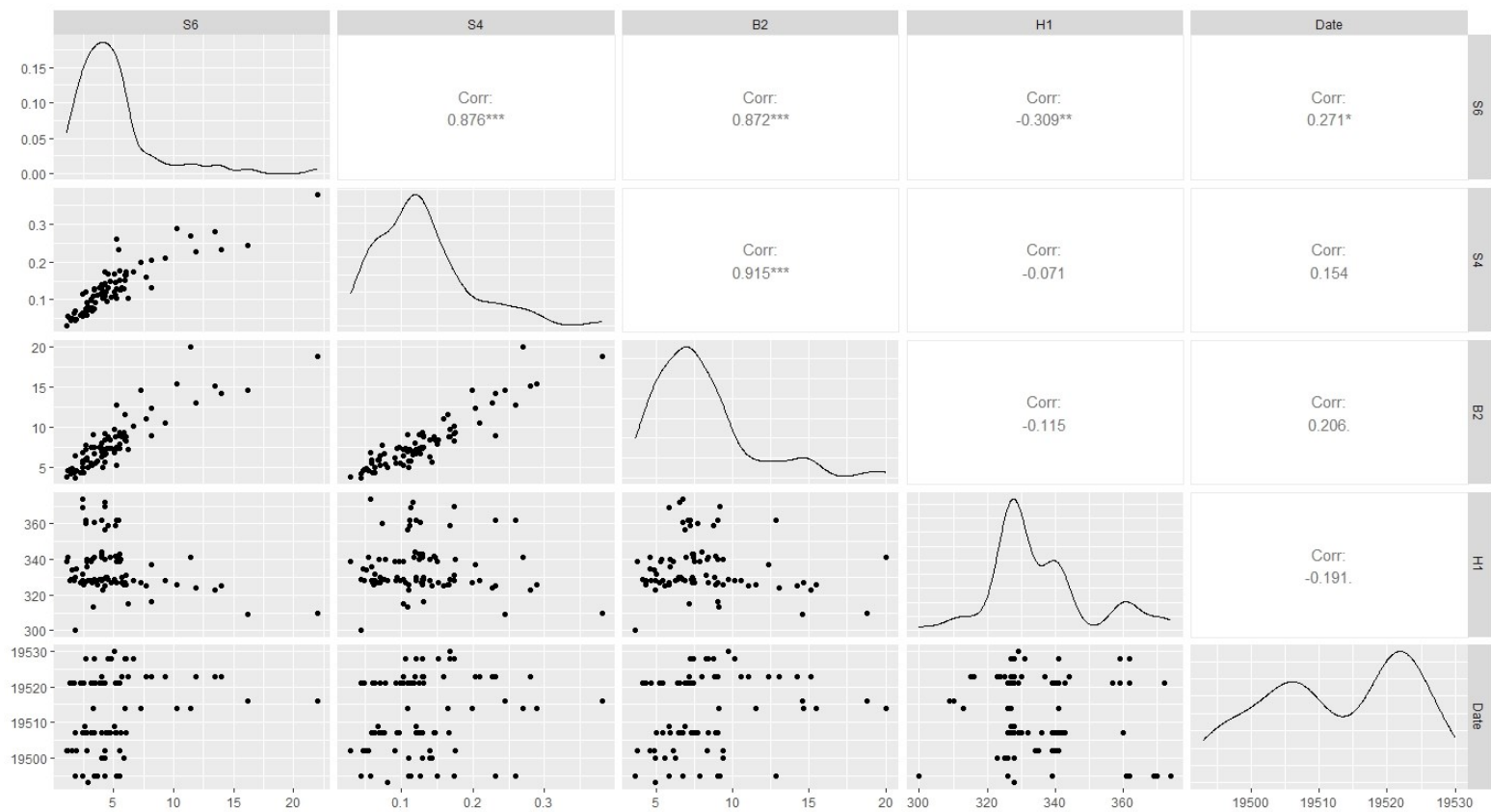


Appendix 1.31 B) Female newts belly total spectrum correlations.

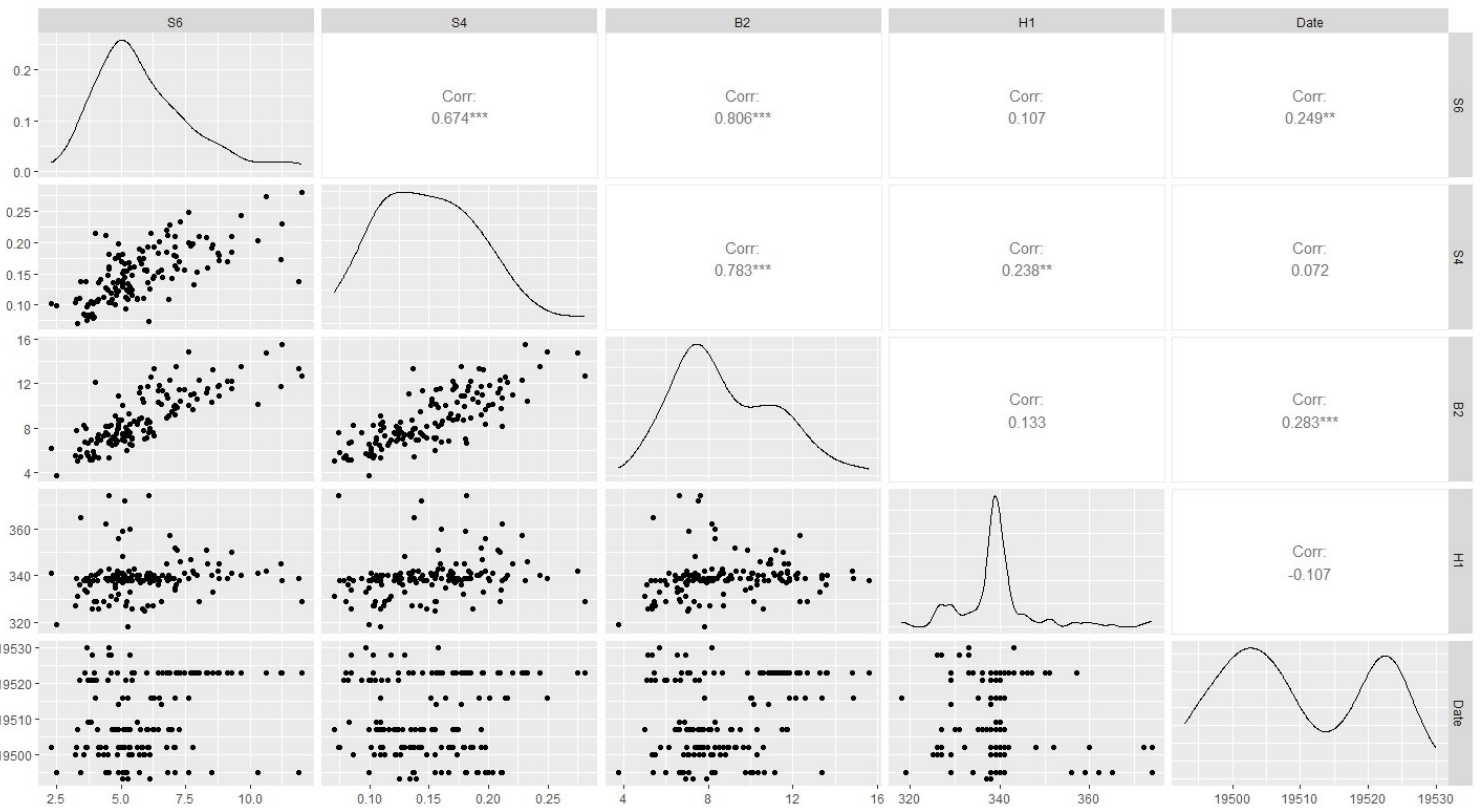
Appendix 1.31 A) Male newts tail UV spectrum correlations.



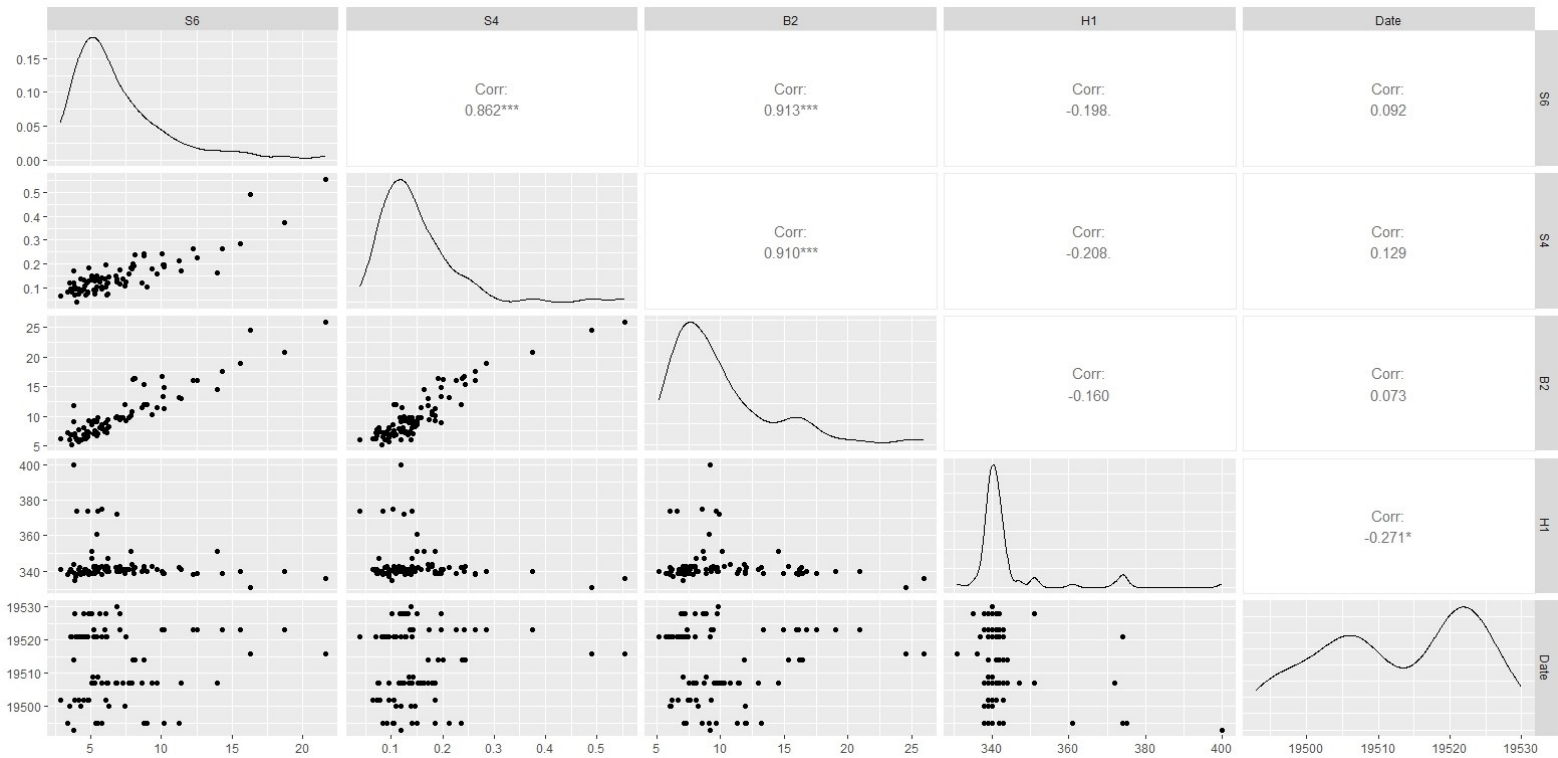
Appendix 1.31 B) Female newts tail UV spectrum correlations.



Appendix 1.31 A) Male newts belly UV spectrum correlations.



Appendix 1.31 B) Female newts belly UV spectrum correlations.







Appendix 2. Full list of linear mixed model including all response variables of body size, belly dot patterns and colorimetric. Water color and Habitat types (Temporary and Permanent) were used as independent variables in all models. Site ID was used as a random factor to control the differences in sample size. T value is for gaussian(normal distribution) whereas Z value is for negative binomial two models. In all negative binomial models my Df values are replaced with N values (and few LMM's).

Trait	Variable	Description	Fixed effects	Sex	Df/N	$\beta$	T/Z	P		
<b>Body size</b>	Fulton index	Body condition (Weight divided by SVL <sup>3</sup> )	Intercept	M	6.85	0.028	28.41	<0.001		
			<b>Color</b>		<b>9.178</b>	<b>&lt;-0.0001</b>	<b>-2.521</b>	<b>0.032</b>		
			Habitat Temporary		10.94	0.0025	1.695	0.118		
			Color:Temporary		17.45	<-0.0001	-1.471	0.159		
			Intercept	F	14.51	0.0247	15.99	<0.001		
			Color		19.72	<0.0001	0.041	0.968		
			Habitat Temporary		19.89	0.0041	1.815	0.087		
			Color:Temporary		21.18	<-0.0001	-1.088	0.288		
			Tail length	-	Intercept	M	6.86	4.189	26.70	<0.001
					Color		8.13	-0.001	-1.232	0.252
			Habitat Temporary		9.05	-0.124	-0.549	0.597		
			Color:Temporary		12.72	<0.0001	0.465	0.650		
			Intercept	F	4.82	2.62	27.87	<0.001		
			Color		7.98	<-0.0001	-1.054	0.882		
			Habitat Temporary		8.52	0.303	1.522	0.164		
			Color:Temporary		8.77	<-0.0001	-0.017	0.987		
		Tail height	-	Intercept	M	145	0.311	1.970	0.049	
				Color		145	<0.0001	-0.548	0.584	
				Habitat Temporary		145	0.097	0.260	0.795	
				Color:Temporary		145	-0.002	-0.550	0.582	
			Intercept	F	83	-0.284	-1.785	0.074		
			Color		83	-0.0001	-0.349	0.727		
			Habitat Temporary		83	-0.004	-0.013	0.990		
			Color:Temporary		83	0.0006	0.279	0.780		
<b>Belly dot patter</b>	Dots	Number of dots in belly surface	Intercept	M	5.81	20.52	19.15	<0.001		
			Color		8.86	0.008	1.243	0.246		



<b>n</b>					<b>Habitat Temporary</b>	<b>12.15</b>	<b>5.49</b>	<b>3.197</b>	<b>0.008</b>	
					<b>Color:Temporary</b>	<b>21.84</b>	<b>-0.036</b>	<b>-3.155</b>	<b>0.005</b>	
					Intercept	F	84	41.62	7.855	<0.001
					Color		84	0.016	0.446	0.657
					Habitat Temporary		84	0.465	0.052	0.959
					Color:Temporary		84	0.037	0.716	0.476
	Dots total area	Total area of all belly dots			Intercept	M	11	139442	5.957	<0.001
					Color		13.02	-149.07	-1.138	0.276
					Habitat Temporary		14.45	23812	0.705	0.492
					Color:Temporary		20.07	-29.71	-0.144	0.887
					Intercept	F	11.99	84761	3.562	0.004
					Color		16.45	-41.34	-0.288	0.777
					Habitat Temporary		16.59	7300	0.211	0.836
					Color:Temporary		18.30	398.18	1.988	0.062
	Solidity	Average solidity of ventral dots			Intercept	M	11.00	139442	5.957	<0.001
					Color		13.02	-149.07	-1.138	0.276
					Habitat Temporary		14.45	23812	0.705	0.492
					Color:Temporary		20.07	-29.71	-0.144	0.887
					Intercept	F	11.99	84761	3.562	0.004
					Color		16.45	-41.34	-0.288	0.777
					Habitat Temporary		16.59	7300	0.211	0.836
					Color:Temporary		18.30	398	1.988	0.062
	Dot/Belly surface	Number of dots divided by belly surface			Intercept	M	7.36	12.47	10.59	<0.001
					Color		9.20	0.012	1.823	0.100
					Habitat Temporary		10.51	2.101	1.217	0.250
					Color:Temporary		15.62	-0.022	-2.089	0.053
					Intercept	F	11.78	25.29	9.414	<0.001
					Color		19.97	-0.002	-0.111	0.912
					Habitat Temporary		21.94	-6.38	-1.490	0.150
					Color:Temporary		20.57	0.005	0.203	0.841
<b>Colori metric</b>	B2_Total_Belly	Mean brightness in total spectral			Intercept	M	13.95	19.27	11.35	<0.001
					Color		14.24	-0.003	-0.384	0.707

	range	Habitat Temporary		16.49	1.70	0.731	0.475
		Color:Temporary		19.29	-0.006	-0.413	0.684
		Intercept	F	14.36	19.13	6.510	<0.001
		Color		16.11	0.003	0.177	0.826
		Habitat Temporary		15.58	2.66	0.693	0.498
		Color:Temporary		17.05	-0.018	-0.837	0.414
S4_Total_Belly	Spectral purity in total range	Intercept	M	16.06	0.14	5.270	<0.001
		Color		15.50	<-0.0001	-0.048	0.962
		Habitat Temporary		17.19	0.03	0.922	0.369
		Color:Temporary		18.15	<-0.001	-0.831	0.417
S4(log)_Total_Belly		Intercept	F	14.94	-1.758	-7.201	<0.001
		Color		17.09	-0.001	-0.870	0.396
		Habitat Temporary		16.95	0.285	0.892	0.385
		Color:Temporary		18.24	-0.001	-0.570	0.576
S8_Total_Belly	Chroma in total spectral range	Intercept	M	11.47	2.095	26.69	<0.001
		Color		13.49	<0.001	0.880	0.394
		Habitat Temporary		16.33	-0.118	-1.136	0.272
		Color:Temporary		22.69	<0.001	1.228	0.232
		Intercept	F	13.79	1.787	18.49	<0.001
		Color		17.45	<0.001	1.272	0.220
		Habitat Temporary		17.50	-0.0156	0.119	0.906
		Color:Temporary		19.34	<0.001	0.212	0.834
H1_Total_Belly	The peak wavelength of hue in spectral range	Intercept	M	136	6.508	730	<0.001
		Color		136	<0.001	0.832	0.405
		Habitat Temporary		136	<-0.01	-0.288	0.773
		Color:Temporary		136	<-0.001	-0.653	0.514
		Intercept	F	80	6.508	552.9	<0.001
		Color		80	<0.001	1.814	0.697
		Habitat Temporary		80	<-0.01	-0.288	0.773
		Color:Temporary		80	<-0.001	-0.467	0.640
H2_Total_Belly	The wavelength at bmaxned of	Intercept	M	136	5.975	594.3	<0.001
		Color		136	<-0.001	-0.545	0.586

	<b>hue in total spectral range</b>	Habitat Temporary		136	<0.01	0.284	0.776
		Color:Temporary		136	<-0.001	-0.527	0.598
		Intercept	F	80	6.006	390.9	<0.001
		Color		80	<-0.001	-1.306	0.192
		<b>Habitat Temporary</b>		<b>80</b>	<b>-0.489</b>	<b>-1.962</b>	<b>0.0498</b>
		Color:Temporary		80	<0.001	1.765	0.078
B2_Total_Tail	Mean brightness in total spectral range	Intercept	M	130	8.785	15.00	<0.001
		Color		130	<0.01	0.903	0.368
		Habitat Temporary		130	-0.33	0.316	0.753
		Color:Temporary		130	-0.01	1.335	0.184
		Intercept	F	13.52	10.78	6.612	<0.001
		Color		20.34	0.001	0.146	0.886
		Habitat Temporary		21.14	2.426	1.035	0.312
		Color:Temporary		22.59	-0.008	-0.578	0.569
S4_Total_Tail	Spectral purity in total range	Intercept	M	6.56	0.107	7.475	<0.001
		Color		8.48	<-0.001	-0.202	0.844
		Habitat Temporary		10.74	0.015	0.712	0.492
		Color:Temporary		16.72	<-0.001	-0.432	0.671
		Intercept	F	12.96	0.120	3.458	0.004
		Color		16.20	<-0.001	-0.083	0.934
		Habitat Temporary		16.21	0.073	1.564	0.137
		Color:Temporary		17.91	<-0.001	-0.996	0.33
S8_Total_Tail	Chroma in total spectral range	Intercept	M	12.00	1.619	10.51	<0.001
		Color		13.18	<0.001	0.877	0.396
		Habitat Temporary		15.64	-0.050	-0.233	0.819
		Color:Temporary		20.14	<0.001	0.363	0.720
		Intercept	F	11.98	1.571	15.73	<0.001
		Color		18.99	<0.001	1.311	0.205
		Habitat Temporary		19.85	0.025	0.173	0.865
		Color:Temporary		20.84	<-0.001	-0.769	0.451
H1_Total_Tail	The peak wavelength of hue in	Intercept	<b>M</b>	136	6.422	321.5	<0.001
		<b>Color</b>		<b>136</b>	<b>&lt;0.001</b>	<b>2.010</b>	<b>0.044</b>

	<b>spectral range</b>	Habitat Temporary		136	0.043	1.194	0.232
		Color:Temporary		136	<-0.001	-1.654	0.098
		Intercept	F	80	6.430	161.7	<0.001
		Color		80	<0.001	1.064	0.287
		Habitat Temporary		80	-0.027	-0.423	0.673
		Color:Temporary		80	<-0.001	-0.007	0.994
H2_Total_Tail	The wavelength at bmaxned of hue in total spectral range	Intercept	M	136	5.946	584.1	<0.001
		Color		136	<-0.001	-0.028	0.978
		Habitat Temporary		136	-0.012	-0.635	0.526
		Color:Temporary		136	<0.001	0.292	0.770
		Intercept	F	80	5.955	380.3	<0.001
		Color		80	<-0.001	-0.241	0.810
		Habitat Temporary		80	-0.0197	-0.780	0.435
		Color:Temporary		80	<0.001	0.761	0.447
B2_Visual_Bell y	Mean brightness in visual spectral range	Intercept	M	13.38	22.91	11.74	<0.001
		Color		14.05	-0.004	-0.334	0.743
		Habitat Temporary		16.43	1.522	0.568	0.577
		Color:Temporary		20.00	-0.004	-0.253	0.803
		Intercept	F	13.67	21.91	6.844	<0.001
		Color		15.75	0.006	0.348	0.732
		Habitat Temporary		15.63	2.292	0.545	0.563
		Color:Temporary		16.88	-0.018	-0.745	0.466
S8_Visual_Bell y	Chroma of visual spectral range	Intercept	M	11.74	1.764	37.13	<0.001
		Color		14.35	<0.0001	0.885	0.391
		Habitat Temporary		17.60	-0.057	-0.836	0.415
		Color:Temporary		25.39	<0.0001	0.946	0.353
		Intercept	F	11.79	1.538	25.43	<0.001
		Color		16.61	<0.0001	1.155	0.264
		Habitat Temporary		17.00	0.055	0.657	0.520
		Color:Temporary		18.74	<-0.0002	-0.343	0.736
H2_Visual_Bell y	The wavelength at bmaxned of	Intercept	M	136	6.020	345.4	<0.001
		Color		136	<-0.001	-0.295	0.768

	<b>hue in visual spectral range</b>	Habitat Temporary		136	-0.024	0.755	0.450
		Color:Temporary		136	<-0.001	-0.928	0.353
		Intercept	F	80	6.006	393.6	<0.001
		Color		80	<-0.001	-0.097	0.923
		Habitat Temporary		80	<-0.01	-0.91	0.927
		Color:Temporary		80	<0.001	0.009	0.993
B2_Visual_Tail	Mean brightness in visual spectral range	Intercept	M	136	9.333	13.14	<0.001
		Color		136	0.006	1.419	0.158
		Habitat Temporary		136	0.103	0.081	0.936
		Color:Temporary		136	0.014	1.518	0.131
		Intercept	F	14.57	11.54	6,323	<0.001
		Color		22.60	0.005	0.444	0.662
		Habitat Temporary		23.57	2.168	0.815	0.423
		Color:Temporary		24.67	-0.008	-0.513	0.612
S8_Visual_Tail	Chroma of visual spectral range	Intercept	M	12.66	1.445	12.29	<0.001
		Color		14.20	<0.001	0.841	0.414
		Habitat Temporary		16.92	<0.01	0.231	0.820
		Color:Temporary		22.27	<-0.001	-0.063	0.950
		Intercept	F	13.00	1.445	20.96	<0.001
		Color		21.54	<0.0001	1.036	0.312
		Habitat Temporary		22.60	0.062	0.606	0.550
		Color:Temporary		23.00	<-0.0001	-1.051	0.304
H2_Visual_Tail	The wavelength at bmaxned of hue in visual spectral range	Intercept	M	133	6.093	98.63	<0.001
		Color		133	<0.0001	0.008	0.993
		Habitat Temporary		133	-0.109	-1.271	0.204
		Color:Temporary		133	<0.001	0.539	0.590
		Intercept	F	78	5.971	157.1	<0.001
		Color		78	<0.0001	1.836	0.066
		Habitat Temporary		78	0.022	0.362	0.712
		Color:Temporary		78	<-0.0001	-0.587	0.557
B2_UV_Belly	Mean brightness in UV spectral	Intercept	M	134	2.28	22.90	<0.001
		<b>Color</b>		<b>134</b>	<b>-0.001</b>	<b>-1.961</b>	<b>0.049</b>

	range	Habitat Temporary		134	0.198	1.347	0.178
		Color:Temporary		134	-0.001	-1.011	0.312
		Intercept	F	78	2.423	18.34	<0.001
		Color		78	-0.001	-1.403	0.161
		Habitat Temporary		78	0.265	1.373	0.170
		Color:Temporary		78	-0.001	-1.006	0.314
H1_UV_Belly	The peak wavelength of hue in UV range	Intercept	M	140	5.841	547	<0.001
		<b>Color</b>		140	<-0.001	-1.078	0.281
		Habitat Temporary		140	<-0.001	-1.124	0.261
		Color:Temporary		140	<0.001	0.334	0.739
		Intercept	F	84	5.836	381.9	<0.001
		Color		84	<0.001	0.505	0.614
		Habitat Temporary		84	<-0.001	-0.698	0.485
		Color:Temporary		84	<0.001	0.285	0.776
B2_UV_Tail	Mean brightness in UV spectral range	Intercept	M	10.34	6.393	7.053	<0.001
		Color		11.17	-0.003	-0.543	0.598
		Habitat Temporary		13.22	1.157	0.372	0.372
		Color:Temporary		16.74	<-0.001	0.924	0.914
		Intercept	F	14.80	7.769	4.175	<0.001
		Color		17.12	-0.005	-0.427	0.675
		Habitat Temporary		17.00	3.502	1.430	0.170
		Color:Temporary		18.37	-0.010	-0.739	0.495
H1_UV_Tail	The peak wavelength of hue in UV range	Intercept	<b>M</b>	6.48	341.7	71.22	<0.001
		<b>Color</b>		7.51	-0.03	-1.11	0.301
		<b>Habitat Temporary</b>		<b>9.12</b>	<b>-16.79</b>	<b>-9.12</b>	<b>0.034</b>
		Color:Temporary		12.63	0.081	12.63	0.077
		Intercept	F	14.45	337.78	51.78	<0.001
		Color		20.53	-0.0178	-0.444	0.661
		Habitat Temporary		21.09	-16.636	-1.809	0.847
		Color:Temporary		22.96	0.091	1.676	0.107

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Appendix 3. Correlation matrix between forest age, water color and DO%. Forest age was calculated from 50m cell grid (fishnet) using mean value from original 5m raster.

