

The impact of biotic and abiotic variations in different waterbodies on Odonata species richness and abundance.



Bachelor Thesis:  
A collaboration between Halmstad University and The  
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## Abstract:

Flying insect biomass has decreased regionally by up to 75% in the last 30 years alone, according to Hallmann et al (2017). Therefore, it is important to determine the qualities of successful breeding habitats to stop this decline. Odonata have been selected as the test subject as odonates are considered good bioindicators and, in addition odonates also offer easy collection and identification. This research focused on the biotic and abiotic factors of wetlands areas offering potential Odonata breeding sites to determine which factors or limits are needed in waterbodies for successful breeding and compare whether different sizes of wetlands impact abundance and species richness. By sampling 3 different habitat types in a river near Halmstad, Sweden called the river Trönningeån, Odonata abundance, and species composition along with abiotic, and abiotic habitat variables was determined. With this information I determine the habitat requirements dragonflies for successful ovipositioning and survival. It was found that aquatic vegetation was the most important factor for influencing Odonata abundance, and that more abiotic information is needed to determine the limits to successful breeding. With regard to waterbody size, it seems that smaller ponds had higher abundance and species richness, however if you go by the diversity indexes this difference between large wetlands is small. was also found that odonates in this region emerge from diapause in March rather than April /May, which is much earlier than previously thought. This information could aid in habitat management, protection, reconstruction, or creation to fulfill these requirements to counteract this decline in biomass and ensure a stable ecosystem in the future.

**Keywords:** Biodiversity, Odonata, Trönningeån, ovipositioning, wetlands, management, protection.

## Sammanfattning:

Flygande insektsbiomassa har minskat med 75% enbart under de senaste 30 åren.. Därför är det viktigt att bestämma egenskaperna hos framgångsrika förökningshabitat för att stoppa denna nedgång. Odonata har valts ut som testorganism eftersom odonater anses vara bra bioindikatorer och odonater kan också enkelt insamlas och identifieras. Denna forskning kommer att fokusera på de biotiska och abiotiska faktorerna i våtmarksområden som är potentiella Odonata förökningsställen för att bestämma vilka faktorer eller gränsvärden som behövs i vattendrag för framgångsrik förökning. Jag jämför också storlek på habitatet påverkan på antal Odonata och deras artrikedom. Genom att ta prov i 3 olika livsmiljötyper för Odonataantal, och artsammansättning tillsammans med abiotiska och abiotiska livsmiljövariabler, syftade jag att bestämma livsmiljöns egenskaper för framgångsrik reproduktion och överlevnad. Det visade sig att vattenväxtlighet var den viktigaste faktorn för Odonataantal, och att mer information om abiotiska faktorer behövs för att bestämma kraven för framgångsrik förökning. När det gäller storleken på vattendraget verkar det som att mindre dammar hade högre överflöd och artrikedom, men om man går efter mångfaldsindexen är skillnaden mellan stora våtmarker liten. Jag fann att odonater i denna region vaknade från vintervilan tidigare än man tidigare trott. Denna information kan hjälpa till att skydda vattenlevande insekters livsmiljöer, restaurering av viktiga akvatiska habitat, och att motverka den minskning av insekternas biomassa som vi i nuläget bevittnar och framförallt att säkerställa friska ekosystem för framtiden.

**Nyckelord:** Biodiversitet, Odonata, Trönningeån, ovipositionering, våtmarker, förvaltning, skydd.

## Introduction:

*“Men all around were clearing available land... Wherever the trees fell the moisture dried, the creeks ceased to flow, the river ran low, and at times the bed was dry. Dragonflies could not hover over dry places and butterflies became scarce in proportion to the flowers” (Stratton-Porter, 1909)*

Flying insect biomass has decreased by roughly 75% in the last 30 years (Hallmann et al. 2017). This is truly alarming, as biodiversity and complex trophic level structures are crucial to maintaining healthy ecosystems (Raffaelli and Hall 1996; Gamfeldt, et al. 2008). The quotation above illustrates that the connection between habitat destruction and the decline of flying insects has been noted for over a century and reveals that the situation is more severe than these recent statistics indicate. Consequently, constructing, rehabilitating and protecting existing habitats which can support diverse ranges of insect taxa, and subsequent monitoring of these sites, is essential. Furthermore, understanding the habitat requirements of flying insects for successful reproduction is vital to create suitable breeding habitats to help increase insect populations (Patten et al. 2015). The order of Odonata, including dragonflies and damselflies, has been selected as this thesis' subject to narrow the broad focus of 'flying insects'. So far little study to the habitat needs of Odonata has been achieved, and so the parameters to their specific requirements for successful breeding sites, and the extent of their vulnerability to waterbody degradation in agricultural systems, remain unclear (Tippler et al. 2018).

Odonata are often used as bioindicators for assessing aquatic systems (Chovanec et al. 2014), principally due to odonates sensitivity to habitat structure and pollution (Chovanec and Waringer. 2001; Saha et al. 2014), and reactions to vegetation coverage and type (Foote and Rice, 2005). In addition their abundance, taxonomical recognition and distinct species characteristics ensure that identification and sampling in the field are relatively simple as both adults and larvae (Chovanec and Raab. 1997). Their use as bioindicators makes them an ideal test subject for this thesis, to monitor water quality and the impacts on aquatic ecosystems. However, adult odonates can be very mobile, so the data gathered using solely adult Odonata may be distorted as this life stage does not fully represent the actual residency at a freshwater site (Patten et al. 2015). Hence, using only one life stage of Odonata as bioindicators can be misleading (Patten et al. 2015) or difficult, and impractical (Sato and Riddiford. 2007). Combinations of adult surveys, larval sampling and exuviae together has been suggested give a clearer indication of habitat use and thus water quality conclusions (Patten et al. 2015; Osbor. 2005).

Odonata require aquatic habitats to complete their life cycle. This study will focus 3 differing waterbody types; large wetlands (over 200 m<sup>2</sup>), smaller ponds (30 – 200 m<sup>2</sup>) and the river itself, as successful breeding sites. Wetlands serve a wide variety of functions (Appendix 1) including regulation processes, nutrients recycling and storing, pollutant removal, as well as providing habitats for species breeding and biodiversity (De Groot. 1992). However, before these functions were known, wetlands were regarded as unproductive lands, which spread disease. This lack of information lead to destruction of wetlands, often to create agricultural zones (Zimmerman. 2001). As a result, river systems were impacted, with less filtration and higher sediment loads. Rivers were also misused as mechanisms to remove wastes from urban areas (Kernan, et al. 2011), and many have been manipulated for this purpose, or for other human needs, by straightening channels and removing riparian zones to be replaced with solid walls. These actions have removed precious and fertile floodplains and changed the properties of the river affecting species biodiversity and composition.

Globally, natural wetlands are one of the most vulnerable and threatened habitat types, with a decline of 30%. In Europe alone, up to 50% of wetlands have been destroyed or impacted from 1970 to 2008 (Dixon et al. 2016). Wetlands contain a high diversity of macroinvertebrates and macrophytes, and thus contribute to ecosystem stability and diversity. To compensate for the loss of wetland areas a protection act called the RAMSAR convention, which came into force in 1975, has been put in place which had acted to highlight the importance of wetlands and facilitate the protection and creation of new wetlands (Ramsar Convention Secretariat. 2013). Many new artificial wetlands are being created, often along highways, agricultural and industrial zones to help filter the contaminants to lower pollution reaching rivers. The construction of these filter wetlands has created shallow vegetated areas for denitrification and nitrogen uptake to lower the risk of eutrophication, these areas also permit odonates to breed and forage (Huikkonen et al. 2019).

Better waterbody management in Europe has led to several Odonata species which were previously considered threatened in the 1990s to subsequently recover over time (Kalkman et al. 2007). This is a good indication that Odonata populations can increase when good quality habits are present. LIFE-Goodstream projects in the Halland region in Sweden, aim to improve water quality along the river Trönningeån catchment near the city of Halmstad (LIFE-Goodstream, 2019). LIFE-Goodstream have created several additional wetlands in this agricultural catchment. Construction of these agricultural wetland projects are primarily to filter, pollutants, nutrients and solid matter from the runoff of the adjacent farmlands, thus enhancing water quality and lowering the chance of waterbodies becoming eutrophic. Additionally, however, these wetlands enhance local biodiversity and add aesthetic beauty to the landscape (Tippler et al. 2018). Thus, creation of new wetlands and management and restoration of existing waterbodies will have a positive impact on many aquatic and flying insect abundances, not limited to just the Odonata order.

Biotic and abiotic variables in waterbodies have a huge impact on the ecosystems, including macroinvertebrates and macrophytes within these ecosystems. The success of Odonata eggs and larvae, depend directly on the quality of the water in which these organisms live. Pollutants can cause inhibited growth in larvae, which can reduce the chances of survival due to increased predation. Acidic waters can change feeding behaviours in some odonate species (Corbet, 1999) and may increase metal concentration by 2-5 times compared to a pH of 7 (Johansson et al. 1995). In both lentic and lotic ecosystems(still and flowing waterways) the presence of certain vegetation is very important for Odonata success (Huikkonen et al. 2019) other factors such as water depth and bottom type diversity, water clarity and nutrient levels need to be investigated to establish a full body of understanding (Huikkonen et al. 2019). From the research of Saha et al. (2018), Silva et al. (2018) and Honkanen et al. (2011), pH and oxygen levels have a large effect on breeding sites. In lotic environments (flowing waters) the velocity of the water is the major factor for breeding success (Ribeiro & Uieda 2005; Silva et al. 2018).

Abiotic factors effect Odonata populations by damaging aquatic habitats in a variety of other ways While nitrogen (N) and phosphorus (P) are essential elements of life and growth, having these in excess can be damaging to waterbodies, causing, anoxic environments, increased toxicity and eutrophication which may reduce aquatic species chance of survival (Drewry. et al. 2006). Excess nitrogen specifically creates problems such as nitrification, which leads to an increase in greenhouse gas pollution (nitrous oxide) contributing to the wider problem of climate change, further perpetuating biodiversity loss (Sobota et al. 2013). Electrical conductivity which measures salts in the water, which effect ionic composition changes in the water which may also result in biodiversity loss and possible chronic effects to organisms. Water hardness can relate to other constituents such as

aluminium, iron, zinc, barium and manganese (Sengupta 2013). Total dissolved solids (TDS) also incorporate salt content and organic matter and other minerals in water, depending on the amount and composition of TDS, toxic environments can be created (Weber-Scannell and Duffy. 2007).

This paper will focus on these biotic and abiotic qualities of the existing and constructed water bodies along the river Trönningeån in Halland, Sweden, and how these factors effect Odonata species richness and abundance. By determining the water parameters where odonate larvae are found, a more complete picture of how to adapt existing, or create new wetlands to accommodate Odonata breeding can be created. The abiotic water parameters which will be the focus of this thesis are water colour, temperature, pH, absorbance, dissolved oxygen, salinity and total dissolved solids, and water hardness. These parameters were indicated by Osborn (2005), Silva et al. (2018), Suhaila et al. (2016) and Campos et al. (2016) to influence dragonfly oviposition and larvae growth. The biotic parameters include aquatic vegetation composition, water area, and bottom type as per recommendations by Chovanec et al. (2015), Sato and Riddiford (2007) and Huikkonen et al. (2019) as other important factors to allow emergence and species variation. Currently, there are conflicting results on whether larger or smaller wetlands are best suited to increase species richness (Huikkonen et al. 2019). This study will aim to discover if larger or smaller wetlands are more suitable to help increase Odonata populations. In this study an adult survey of the region will be compared to the larvae sampled to show any discrepancies between adult and larval distribution. The presence of the aquatic Odonata larvae will be the focus for demonstrating that successful breeding has occurred, and to indicate that the sites were not simply roosting or foraging sites of adults, (Patten et al. 2015). This will add more information to the discussion about the appropriate use of different Odonata life stages as bioindicators in field surveys.

## Aim:

The information gathered from this research firstly aims to determine the optimal habitat conditions for Odonata which could be used to give useful scientific guidelines on future wetland projects, restoration projects of existing wetlands or creation of new agricultural runoff sites to primarily ensure the success of the Odonata order. Secondly this research may help other species of flying insects which rely on aquatic habitats during their life cycle to recover, either by directly improving these existing habitats or by providing knowledge about species needs to provide suitable breeding grounds (Patten et al. 2015).

Within this thesis I hypothesise that:

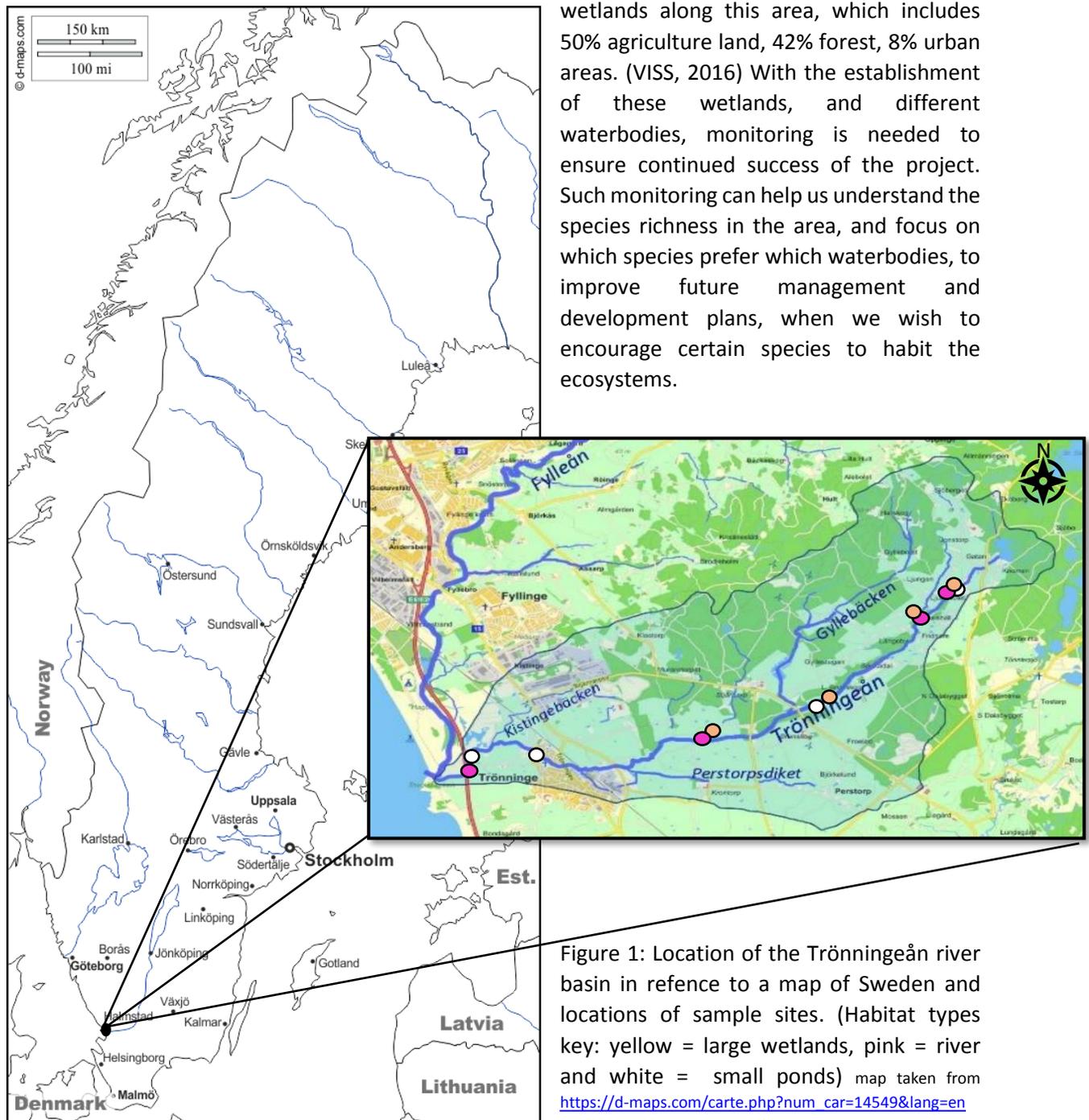
- A higher diversity of Odonata larvae will reside in the larger waterbodies (Chovanec et al 2015)
- There will be a higher abundance of Odonata species in the wetlands (Huikkonen et al. 2019) rather than the rivers (Chovanec et al 2015).
- The percentage of aquatic vegetation cover impact Odonata diversity (Huikkonen et al. 2019)
- The abiotic conditions successful breeding will relate to dissolved oxygen levels (Chovanec et al 2015), pH, and total dissolved solids (Silva et al. 2018).
- The differences between the adult population surveyed compared to the larvae at the sample sites, will show that odonates can use locations differently, indicating the ineffectiveness of only adult Odonata as bioindicators in these locations.

## Materials and methods:

### Location:

The LIFE-Goodstream program is an EU-funded conservation project (LIFE Programme, 2019), existing in Halland, a county of Sweden. Their focus is to improve the ecological status of streams within a heavy agricultural zone, and the project has been operating here since 2015. During this time, the project has constructed amphibian ponds, restored wetlands, removed dispersal barriers in the stream and has implemented strategies to improve the Ecological status of the 11 km river Trönningeån (GoodStream, 2019). Part of their achievements is the creation and monitoring of several

wetlands along this area, which includes 50% agriculture land, 42% forest, 8% urban areas. (VISS, 2016) With the establishment of these wetlands, and different waterbodies, monitoring is needed to ensure continued success of the project. Such monitoring can help us understand the species richness in the area, and focus on which species prefer which waterbodies, to improve future management and development plans, when we wish to encourage certain species to habit the ecosystems.



The different habitat locations along the river Trönningeån, and all sites have been chosen due to size diversity, previous surveys completed in these locations and ease of access. To be studied are the larger (over 200 m<sup>2</sup>) wetlands, smaller ponds (30 – 200 m<sup>2</sup>) and the stream itself. Odonata larvae were collected from the 3 differing waterbody types (large wetland, small pond and stream), at 12 sites (see fig. 1). Many of these wetlands are situated in agricultural catchments constructed on private land with the growth of cereals, and farm-animal raising, with some industry within the catchment zone. The locations were chosen to coincide with surveys of adult dragonflies, which were undertaken by John Stand of LIFE-Goodstream. The information from these adult odonata surveys will give additional information of which species uses the wetland only for reproduction (if there are larvae but no adults) and which use it only for food/foraging as adults (adults but no larvae) or use it for both purposes (both adults and larvae) to give a better of species distribution and locations where the adults find suitable for breeding.

### Study design

The focus of this thesis is to document Odonata species inhabiting different types of waterbodies, by comparing the properties of the habitats, to determine what aspects are most advantageous for successful breeding. A comparative study on Odonata composition in differing waterbodies was undertaken, including 4 large wetlands, 4 small ponds, and 4 locations of the Trönningeån river. Here I measured biotic and abiotic factors of the habitat in relation to previous studies undertaken on Odonata abundance and diversity. Abiotic data was collected on all sites on the 5<sup>th</sup> of March 2020, including abiotic measurements in the field (turbidity, secchi depth, water colour, temperature, pH, absorbance, dissolved oxygen, electrical conductivity, salinity and total dissolved solids, and water hardness). In addition, the percentage coverage of aquatic vegetation was monitored as a biotic factor. I also collected water samples to be analysed for water hardness, nitrate, total N & P, and TDS. Larvae sampling occurred over 3 separate days, 3<sup>rd</sup>, 5<sup>th</sup> and 19<sup>th</sup> of March 2020, with a standardised 45-minute unit effort sampling time allocated to all waterbody sites.

### Study species

Odonata is an abundant order, which includes dragonflies and damselflies, and is found on all continents except for Antarctica (Clausnitzer et al. 2009). Odonates inhabit a wide range of environments including bogs, lakes, brackish estuaries, rivers and temporary waters (Nillson. 1997). Odonata is a well-recognised and studied order, which has a presumed worldwide distribution of around 5,952 species (Saha et al. 2018), with 64 species in Sweden (Dragonflypix, 2020). The IUCN Red List states that 15.3% of global known species are near threatened to critically threatened (IUCN. 2020), highlighting the seriousness of the flying insect decline. Dragonflies are important to aquatic ecosystems, as they act both as prey and predators during their life cycle (Corbet. 2004) and are also often used as bioindicators of habitat health (Kalkman et al. 2007) however there is some uncertainty as to the accuracy of this practice (Patten et al. 2015). A physical description of Odonata adults and larvae can be found in Appendix 2.

The life cycle of Odonata is dependent on the availability of water for successful ovipositioning. Breeding occurs in the air and eggs are often deposited on aquatic plants under the waterline (Matushkina, & Gorb. 2007) certain species needing specific plants to reproduce (Huikkonen et al. 2019) and will hatch within a few weeks (Stoks. 2010). During the larval stage the larvae are solely aquatic (Corbet. 2004) and carnivorous, preying on living organisms, including tadpoles and even small fish (Kalkman et al. 2007). The larvae can take anywhere from a few weeks to 7 years to develop,

depending on the species and habitat conditions, and undergo about 10 moults in this time to allow for growth (Stoks. 2010). At the end of the growth stage, the larvae will crawl up above the waterline, then with the final moult they metamorphose into the adult phase (Kalkman et al. 2007). Once the exoskeleton has hardened the fresh adults emerge leaving behind a shell called an 'exuviae' (which can also be used to identify specific Odonata species). Adults will often disperse away from the breeding site to hunt and gain body mass, during this time they will reach sexual maturity, with the females developing eggs, at which point they will return to the water (Stoks. 2010).

Often between 50%-95 % of larvae do not survive to emerge (Corbet. 2004; Patten et al. 2015) and egg mortality is around 25%, as found in *Pyrrosoma nymphula* (Stoks. 2010). Fish are the primary enemy of Odonata, eating both eggs and larvae which can affect the distribution of some species (Nillson. 1997). Stoks and Córdoba-Aguilar (2010) found that the environmental parameters can influence egg development, such as lower temperatures leading to eggs hatching too early, before full development, thus resulting in smaller hatchling sizes, which can increase predation on the hatchlings, consequently reducing the chance of reaching adulthood. It was found that survival until adulthood was increased with later egg hatching times and so the effects of larval conditions directly correlates to adult fitness and breeding success.

## Field work

At each site the abiotic data was first collected (dissolved O<sub>2</sub>, EC, pH and temperature) using a dissolved oxygen meter model HANNA instruments HI 9146, a conductivity meter model HANNA instruments HI 991301 and a thermometer. The area of the large wetlands and ponds were measured (Huikkonen et al. 2019; Campos et al. 2016) to determine whether the waterbody is considered, large (over 200 m<sup>2</sup>), or small (30 – 200 m<sup>2</sup>), which were used also used as measures of habitat area for the Odonata. Next, the biotic data regarding aquatic macrophyte composition in percent was noted, no specific species were identified as their presence of aquatic vegetation was all that was required for data recording. The observations were done by sight from the shoreline and the coverage was then estimated. Water colour was also observed which often translates well to water quality and eutrophic state of the waterbody (Carlson, 1977) and the bottom type according to mud, visible organic matter and stones was noted.

The temperature was taken twice at each location and the mean determined for accuracy. The pH and EC were measured at the same occasion. When measuring the oxygen levels, the O<sub>2</sub> meter was standardised to be calibrated to 100% in air then immediately put into the water source for exactly 20 seconds (measured on a stopwatch) to give standardised results. Water transparency and depth of each sampling site was measured with a Secchi disk from within 1 m of the shoreline, where the larvae were sampled. A water sample was taken at each site for further analysis of dissolved oxygen and chemical elements such as total nitrogen (TN), total phosphorus (TP), nitrate and calcium.

Samples of larvae were taken in a standardised method for sampling, using a 20 cm oval water net with a mesh size of 2 mm, and swiping the net in a Z pattern 3-4 times, and emptying contents into a white container to sort through the contents of the net to look for samples. The Odonata catch per unit effort was set to 45 minutes at each site. Site F large was resampled two days after the first unsuccessful attempt. All larvae samples were collected in plastic containers labelled with location and site to eliminate confusion and site contamination, to determine species abundance and then fixed in an 80% alcohol solution to be then identified to species level, in the laboratory.

## Laboratory analysis

Chemical analyses were performed under standard conditions. Filtration was used to determine total dissolved solids (TDS), which was weighed to determine the mass of solids in the sample then this was divided by the sample amount in litres. A titration was used to determine the water hardness.

A flow injection analyser (FIAstar 5000 Analyzer) was used to determine the TN, total TP and nitrate. The standards AN 5202 and AN 5201 were followed.

## Statistical analysis

The mean, standard deviation, minimum and maximum of abiotic variables were analysed in SPSS with the data collected, a biodiversity calculator (Biodiversity Calculator. 2020) was also used to determine the Simpson Index and the Shannon Index. These indices help to measure and quantify the commonness or rarity of species in a community (Beals, Gross, and Harrell. 2000). From this data, the evenness was calculated using the division of the log of the species richness to the Simpson's index. These values are represented in Appendix 4 table 8.

Subsequent statistical analysis was performed using the SPSS program, my data was not normally distributed at  $p=0.033$  and  $p=0.037$ . To find if any differences existed between biotic and abiotic variables, a Kruskal-Wallis test (K Independent Samples) was tested with pH, EC (mS), dissolved oxygen (%), aquatic vegetation (%), hardness (mol/L), TN (ppm), TP (ppm) and nitrate (ppm) in regards to species richness, the uncorrelated data was then excluded from further analysis and the similarities were focused in association tests. To find the associations for the data a Spearman rank correlation for non-normally distributed data was used to determine the important correlations between data, which provided the most significant factor effecting abundance and species richness. From this data graphs were created to visually represent the information gathered.

## Results:

### Odonata diversity and abundance in test sites

Encompassing all test sites, 9 different genera were collected in their larval form, with a total number of 351 individuals. Figure 2 shows the larval abundance and species richness of each of the habitat types, where the large wetlands had a species number of 6, and a total abundance of 100, the stream had a species richness of 4, and an abundance of 18, and the small pond a species richness of 7, and an abundance of 233. Overall, the number of genera per site ranged from 1 to 7. The two most abundant taxa were *Coenagrion* and *Enallagma*, representing 66% of the total number of larvae collected. Furthermore, the Simpson's Index (Biodiversity Calculator. 2020) showed that the large waterbodies were marginally more diverse ( $D=0.397$ ) than the small waterbodies ( $D=0.338$ ), and that the river had a very low Odonata diversity ( $D=0$ ) and the abundance ranged from 3 to 119, full data can be found in Table 5 in Appendix 3.

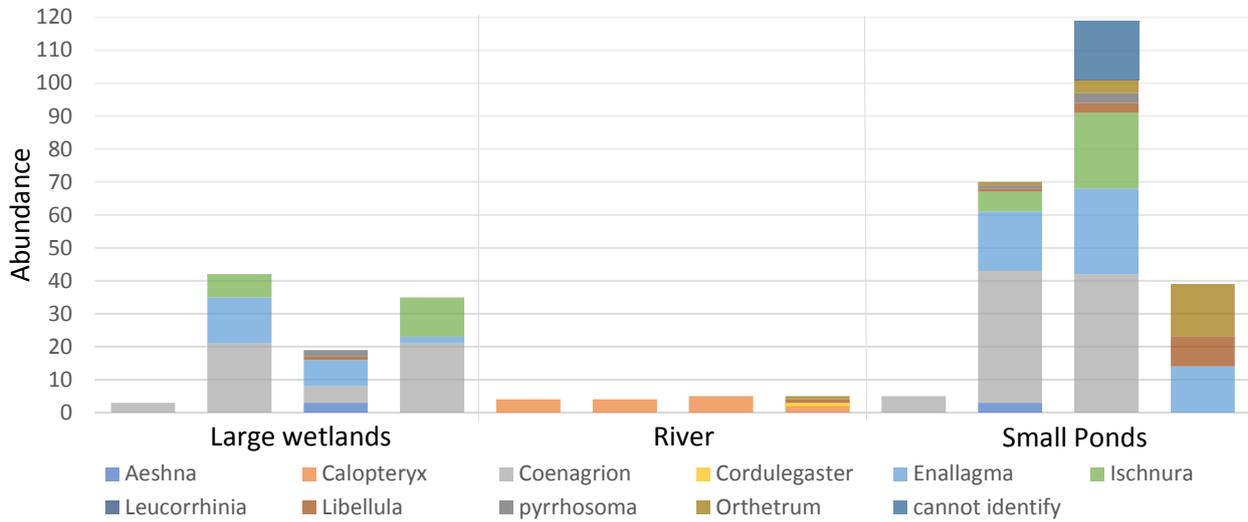


Figure 2: larvae samples abundance in selected waterbody types

### Biotic factors in breeding locations

A Spearman's Correlation was used to find association with the variables, due to the non-normally distributed data. The results of the correlation are pH ( $p = 0.592$ ), hardness ( $p = 0.568$ ), TDS ( $p = 0.091$ ), TN ( $p = 0.566$ ), TP ( $p = 0.486$ ), nitrate ( $p = 0.638$ ), temperature in  $^{\circ}\text{C}$  ( $p = 0.546$ ), EC ( $p = 0.782$ ), dissolved oxygen ( $p = 0.782$ ). My data shows most P values were  $> 0.05$  thus there was no significant relationships between the factors. Only aquatic vegetation correlated significantly with larvae abundance ( $p = 0.008$ ;  $R^2 = 0.52$ ) and with species richness ( $p = 0.004$ ;  $R^2 = 0.53$ ). Figure 3 shows the correlation between Odonata abundance and aquatic vegetation, this indicates that aquatic vegetation has a significant positive correlation, for both species richness and abundance.

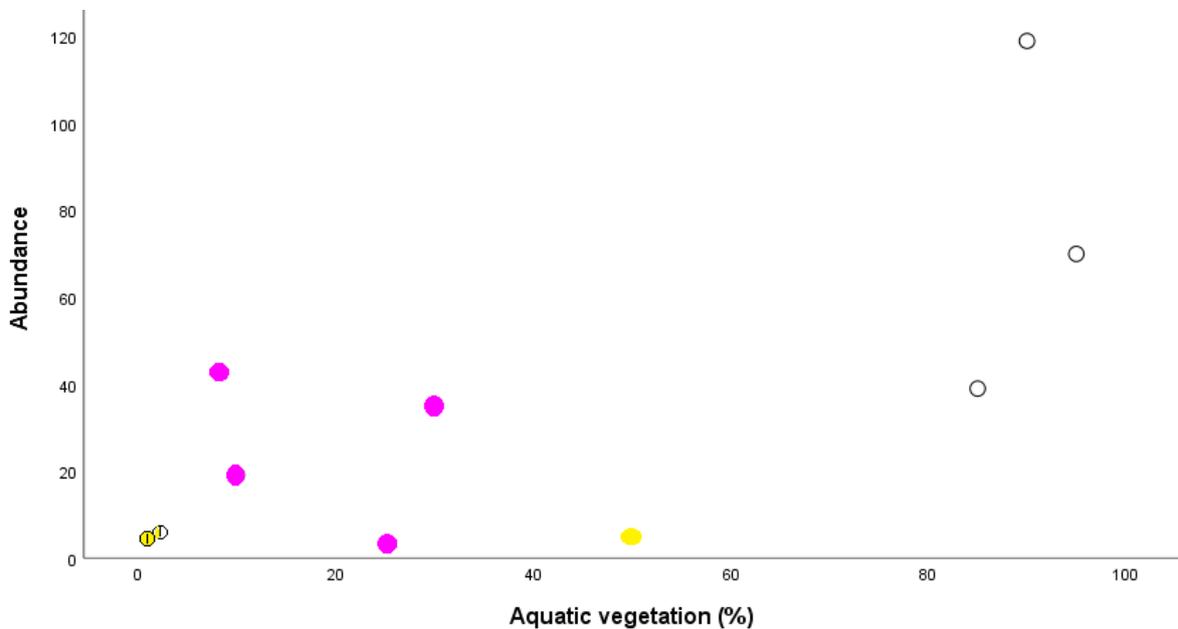


Figure 3: representation of the correlation between aquatic vegetation and abundance (number of individuals) where the yellow represents the river, pink is the large wetlands and the white is for the small ponds.

## Abiotic water variables

The mean and standard deviation of all 12 sites and each waterbody type is shown below in Table 1, which demonstrates that all waterbody types had acceptable water quality, with little abiotic variance between them.

Table 1: The mean,  $\pm$  standard deviation (SD) of abiotic variables of all combined waterbodies and the individual test waterbody types.

Waterbody type		Temperature Celsius	pH	EC (mS/L)	Dissolved Oxygen (%)	Hardness (mmol/L)	TDS (mg/L)	TN (ppm)	TP (ppb)	Nitrate (ppm)
River	mean	5.63	6.73	0.17	84	2.48	21	2.37	0.06	2.65
	SD ( $\pm$ )	0.59	0.45	0.07	7.13	0.5	14.28	0.95	0.09	1.18
Large Wetland	mean	5.23	6.99	0.2	80.05	2.38	32.5	3.30	0.04	2.82
	SD ( $\pm$ )	0.54	0.39	0.06	6.65	0.44	25	4.00	0.05	2.62
Small Pond	mean	6.28	6.46	0.25	86	2.89	71.75	3.19	0.03	2.99
	SD ( $\pm$ )	1.75	0.47	0.11	8.00	0.63	124.20	2.70	0.03	2.64

## Discussion:

My study, in line with several previous studies show the positive relationship between Odonata species richness and abundance with the presence of aquatic vegetation. Further studies show which specific macrophyte type (floating, submerged, emergent) are beneficial for different factors, such as hiding, foraging and breeding (Huikkonen et al. 2019). It was found that both large wetlands and small ponds hosted many Odonata species, often in high abundance and The Trönningeån river system despite this lack of diversity, however, represented two niche species which are uniquely river dependent, the *Cordulegaster boltonii* and the *Calopteryx virgo* which did not occur in wetland environments highlighting the importance of different waterbodies for Odonata species diversity. In addition it was discovered that Odonata larvae are active in these waterbodies earlier than previously thought. Odonata generally enter diapause around the winter months, due to limited photoperiods and cooler temperatures (Flenner, et al. 2010). Non-diapause months have been specifically noted as April and May where development is stimulated by the increase of day length (Corbet, 1956). My results show that this information may be changing due to increasing global temperatures. Warmer weather was noted in the halland region of Sweden, during the sampling year of 2020. A very warm winter, lacking snow cover for any extended period of time, or prolonged ice cover on waterbodies occurred during this season. From my sampling many Odonata taxa were caught in early March when they are generally regarded to be in diapause at this time. Indicating perhaps that temperature has a larger impact than photoperiod, which will likely effect aquatic insect biomass in various ways.

While previous studies by Chovanec et al (2014) state that larger waterbodies have a higher species numbers, my results showed that the effect of habitat area on species richness of Odonata are somewhat contradicting to expectations and previous studies, where there was little difference statistically between species richness and large and small ponds. Perhaps suggesting that microhabitats were more significant than habitat area (Honkanen et al. 2011). Due to the weight that evenness is expressed in the Simpson Index, the result that the large wetlands are the most diverse waterbody, seems to strongly contrast what is shown in figure 2, where, the small pond appears to be the most diverse. however, if we consider only the species richness and abundance of each waterbody

type and disregard evenness, it is clear that the small pond has the highest diversity and abundance. This phenomenon was also strange as the Odonata species were seen to cannibalise each other in during sampling in the close proximity of the sample jars, this behaviour would be expected to occur in smaller areas where competition would be increased. However while this behaviour has been noted in laboratory conditions, and in my own sampling, it is not confirmed if this behaviour occurs in the wild (Suhaila, et al. (2016)

It was assumed that the large wetlands would have an increased species richness due to the relative safety of these systems as there would be a lack of large predators in relation to the species–area relationship theory (Huikkonen et al. 2019), however in contrast to this assumption fish were located at 2 of the wetland sites (location C ‘large Wetland’ and A ‘small pond’ – See appendix 4) and may have effected the number of individuals or the number of species present as both sites only housed the very common and adaptable *Coenagrion puella* with the low number of 4-5 individuals sampled in the 45 minute unit effort time. The presence of the fish in the wetlands could be natural or as a result of flooding in the weeks before sampling, so further testing would be needed to determine if the fish are permanent fixtures in these waterbodies. Another predator was found at site ‘A small pond’ a *Ranatra linearis* or Water Stick Insect, which preys on dragonfly larvae, as stated this site was lacking in both abundance and richness. As Odonata are predators ((Clausnitzer et al. 2009) prey was discovered at all sites while sampling including worms, snails, *Corixa* species, crustaceans and mayfly larvae representing plentiful resources for the dragonflies and damselflies.

Adult surveys had previously been compiled in 4 of the 6 locations of this study, these results showed that this region had a total of 19 different species. Many of these were found in multiple locations as shown in Table 6 in the Appendix 3. This information showed that there were 5 species which are unique to certain locations, which are all found around large wetlands, however due to the mobility of Odonata, this is not solely reflective of true distribution, as many large waterbodies were in close proximity to the smaller ponds. The most common adult species, which was located at 5 sites is the *Sympetrum striolatum* or the common darter this species can inhabit different waterbody types including lakes, ponds, and slow-moving rivers. (White and Smith. 2015). While in many studies Odonata has been praised as a good bioindicator, many reservations have been raised about their suitability in this regard. Appendix 3 indicates the differences found when each location was surveyed for solely adults compared to when only larvae was sampled.

It was discovered that many of the adult dragonflies which were seen in these previous surveys were not located as larvae at the sites. While this discrepancy is maybe due to the life cycles of individual species, such as the *Sympetrum* sp which exist as only eggs during winter, the inconsistencies can be interpreted that adults occupy a broad geographical range, stating clearly that adult presence does not indicate breeding (Patten et al. 2015), and caution should be used for Odonata being used for bioindicators. It should be noted that some factors may have affected the richness of samples such as the time of year that sampling occurred (early March 2020) during the expected diapause stage, and with the possibility of some nocturnal behaviours, and the inability to sample from the middle of the larger wetlands where microhabitats may be preferred indicate that the samples taken may not fully representative of the total Odonatan community in these waterbodies (Sato & Riddiford. 2007). Further discrepancies were the discovery of an additional species, the *Pyrrhosoma nymphula*, which was located at various sites but was not identified during the adult survey. These factors highlight the need to monitor all stages of the Odonata lifecycle at a location to truly determine the use and quality of the site.

While it has been reported in several studies that certain abiotic factors effect Odonata breeding sites, data collected in this report cannot outline the specific limits that Odonata has in regards to successful ovipositioning, as the abiotic variables of the water at my sample sites do not vary beyond significant levels. For example, all the pH results are within the optimal limits for freshwater which is noted at a pH 6-8 for life to thrive (Fondriest. 2020), except for location F site 'small pond', where the Ph was at 5.79, which is just below the optimal range, but this had little effect on abundance, with the site having the 4<sup>th</sup> highest abundance (39), but with lower species richness (3). Electrical conductivity, representing salinity, is also well within the freshwater range for human consumption (0 to 0.8 mS/m) (Mary River Catchment Coordinating Committee 2018). All waterbodies had water which is considered to be 'hard' water (between 1.79 – 3.75 mmol/l) referring to the calcium and magnesium carbonate content in the water, with the slight exception of location D 'large Wetland', with just under at 1.75 mmol/L. Sweden and most of Europe is known to have naturally hard water and this does not cause any environmental or human health effects. Acceptable levels of total dissolved solids (TDS) are under 500 mg/L (Sheffer. 2003) which encompasses my results. However, the smaller ponds have significantly higher TDS than the other wetland types, but still has not affected the Odonata abundance or species richness.

The study by Huikkonen et al (2019), stated the importance of water transparency in relation to species richness and aquatic vegetation. While this may have an indirect effect on odonate species richness by influencing the amount of light which enables aquatic vegetation my results did not support the connection between richness and TDS. As Odonata were found in all waterbodies regardless of turbidity with no specific correlations being discovered for abundance and water clarity in terms of TDS ( $R = -0.298$ ), specific requirements are not clear. However, the site with the worst water quality, location C 'Large Wetland', did have low abundance and richness. Further studies have noted that odonates are attracted polarised light this is shown with odonates often ovipositioning into oilspills (Horváth. et al. 1998) which may indicate, that the only attracting factor for Odonata is polarization, and that water quality only determines the overall success of the eggs and larvae to survive until adulthood.

The EPA's standard for acceptable TN, inclusive of nitrate levels for ecosystems is 0.1 to 4 mg/L (U.S. EPA, 2002) in waterbodies with heavy human influence, where in contrast in fresh unpolluted water is usually below 1mg/l, (EPA. 2020). The nitrate concentration in most of my sites is on the high end of acceptable, and is most likely due to the surrounding agricultural fields in this area, 2 sites go far beyond these acceptable limits with 6.418 (D small) and 6.087 (F large), this factor has not affected the abundance or richness at either site. However, the Spearman's correlation revealed that there was no significance between these elevated factors and species abundance. TP has no strict standard, but a suggested limit is from 0.01 to 0.075 mg/L, depending on the ecoregion (U.S. EPA, 2002) only 2 sites exceed this, including location A 'river' with 193 ppm, and F 'large Wetland' at 0.11ppm (EPA. 2020) Despite some of the abiotic factors occurring outside the normal or recommended levels, none of these factors contributed any changes with larvae abundance or Odonata species richness, thus no clear limits to abiotic factors can be determined with this data.

## Conclusion:

The percentage of coverage of aquatic vegetation correlated to the species diversity and abundance of Odonata (accepting hypothesis 3). A slightly higher diversity of larvae was discovered in the larger wetlands rather than the small ponds (accepting hypothesis 1) and both the wetland types had a higher diversity of Odonata than the river (accepting hypothesis 2), however my study could not prove that DO, pH or TDS were factors in Odonata breeding sites (not validating hypothesis 4). Due to the differences in the Odonata adult and larvae compositions in the test region, I believe that odonates can be used as bioindicators only when the presence of larvae is found to prove breeding has occurred and that the water quality is good enough to support the larval growth (accepting hypothesis 5). Overall, the presence of aquatic plant species appeared to be the most important factor for species richness. In regards to the river environment, slower water and more vegetation gave a higher diversity shown in location F 'River', in contrast all other river sites has a higher velocity of flow, which only supported *Calopteryx virgo*. In addition, samples should be taken both in winter and in summer for more conclusive information on species distribution in further studies. Exuvia should also be collected when possible, to prove that the larvae were able to fulfil their complete lifecycle. This would give a clearer indication that these conditions are required to increase the Odonata populations.

I believe that we can determine with this study, that while waterbodies may have different abiotic values and ranges, many Odonata species can successfully breed if these habitats are within normal ranges as long as adequate aquatic plants are present. Additionally, fewer predators in the ecosystem will mean a greater abundance and diversity of Odonata. It is also clear that man-made wetlands are capable of supporting various species of Odonata and other aquatic species, both helping to increase insect biomass and also helping to purify water before it enters the rivers and oceans. These wetlands have a clear benefit to the aquatic ecosystem and the environment as a whole and should be encouraged in all heavy agricultural areas as well as industrial or modified landscapes. Having current and informed knowledge on the best methods to create these wetlands with diversity as a key motivator will help to increase all species which depend on water to complete their life cycle. This includes Odonata, who become an integral part of the aquatic systems with their predatory behaviour.

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## Appendix

### Appendix 1- Properties of Wetlands

Regulation	Protection against harmful cosmic influences Regulation of the local and global energy balance Regulation of the chemical composition of the atmosphere Regulation of the chemical composition of the oceans Regulation of the local and global climate (including the hydrological cycle) Regulation of runoff and flood prevention (watershed protection) Water-catchment and groundwater recharge Prevention of soil erosion and sediment control Formation of topsoil and maintenance of soil fertility Fixation of solar energy and biomass production Storage and recycling of organic matter Storage and recycling of nutrients Storage and recycling of human waste Regulation of biological control mechanisms Maintenance of migration and nursery habitats Maintenance of biological (and genetic) diversity
Carrier	Human habitation and (indigenous) settlements Cultivation (crop growing, animal husbandry, aquaculture) Energy conversion Recreation and tourism Nature protection
Production	Oxygen Water (for drinking, irrigation, industry, etc.) Food and nutritious drinks Genetic resources Medicinal resources Raw materials for clothing and household fabrics Raw materials for building, construction, and industrial use Biochemicals (other than fuel and medicines) Fuel and energy Fodder and fertilizer Ornamental resources
Information	Aesthetic information Spiritual and religious information Historic information (heritage value) Cultural and artistic inspiration Scientific and educational information

Figure 4: *Wetland Ecology: Principles and Conservation*. Wetland properties. (Keddy. 2000).

## Appendix 2: Further information on Odonata



Figure 5: *Aeshna* sp.



Figure 6: *coenagrion puella*

The adults of Odonata, represented in figure 5, are characterised by their often-colourful long slender abdomen, long double set of wings, proportionally large eyes (Saha et al. 2018), strong mandibles and long thin spiny legs (Ab Hamid et al. 2016). Dragonflies are carnivorous, visual predators (Suhaila et al. 2016). They are fast, strong and manoeuvrable aerial fliers, necessary for hunting, reproduction and eluding predators (Saha et al. 2018). Many species have a limited habitat range, however some are capable of long distance dispersal as their bodies are desiccation-resistant (Patten et al. 2015) while others are considered habitat specialists, occupying niche locations such as mountain bogs, seepage areas, and waterfall, with some species can also tolerate brackish water (Kalkman et al. 2007).

The larvae are diverse in characteristics due to the sub orders of Odonata, Anisoptera (dragonfly) shown in figure 6 and Zygoptera (damselfly) in figure 7. The larvae live underwater and breathe through gills. Damselfly larvae have the gills in the form of three long appendages extending from the tail end of their abdomen; dragonflies lack these appendages, and have internal rectal gills (CSIRO. 2004), long segmented abdomens, and wing sheets. They hunt with their enlarged, hinged labium (jaw like structure shown in figure 8), which is used as an extendable grasping which shoots out to spear and capture prey, figure 9 (Ab Hamid et al. 2016).



Figure 7 and 8: hinged labium, withdrawn and extended.

Reference: Ab Hamid, Suhaila & Salmah, Md & Nurul Huda, A. (2016). 'Composition and distribution of Odonata larvae and its relationship with physicochemical water quality in northern peninsular Malaysia.' *Malaysian Journal of Science*. 35(issue 2). 198-209.

Images: Taken by Sara Bischoff

## Appendix 3: Adult and larvae surveys

Table 5: Odonata larvae collected at each site.

waterbody type	<i>Aeshna</i>	<i>Calopteryx</i>	<i>Coenagrion</i>	<i>Cordulegaster</i>	<i>Enallagma</i>	<i>Ischnura</i>	<i>Leucorrhinia</i>	<i>Libellula</i>	<i>pyrrhosoma</i>	<i>Orthetrum</i>	cannot identify
Large	0	0	3	0	0	0	0	0	0	0	0
Large	0	0	21	0	14	7	0	0	0	0	0
Large	3	0	5	0	8	0	0	1	2	0	0
Large	0	0	21	0	2	12	0	0	0	0	0
River	0	4	0	0	0	0	0	0	0	0	0
River	0	4	0	0	0	0	0	0	0	0	0
River	0	5	0	0	0	0	0	0	0	0	0
River	0	2	0	1	0	0	0	1	0	1	0
Small	0	0	5	0	0	0	0	0	0	0	0
Small	3	0	40	0	18	6	0	1	1	1	0
Small	0	0	42	0	26	23	0	3	3	4	18
Small	0	0	0	0	14	0	0	9	0	16	0

Table 6: Adult species composition of testing region

Location / Species	<i>Aeshna cyanea</i>	<i>Aeshna grandis</i>	<i>Aeshna mixta</i>	<i>Anax imperator</i>	<i>Calopteryx virgo</i>	<i>Coenagrion puella</i>	<i>Cordulegaster boltonii</i>	<i>Enallagma cyathigerum</i>	<i>Ischnura elegans</i>	<i>Lestes sponsa</i>	<i>Leucorrhinia pectoralis</i>	<i>Libellula depressa</i>	<i>Libellula quadrimaculata</i>	<i>Orthetrum cancellatum</i>	<i>Orthetrum coerulescens</i>	<i>Sympetrum danae</i>	<i>Sympetrum sanguineum</i>	<i>Sympetrum striolatum</i>	<i>Sympetrum vulgatum</i>
A																			
C Large	X		X				X									X		X	
D Large	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
D Small		X						X	X									X	X
E Large	X	X	X				X	X		X	X	X	X			X	X	X	X
E Small	X	X	X					X		X		X	X			X	X	X	X

Appendix 4: Site specific variables

Table 7: Abiotic variables at sample sites

Location	waterway type	Temperature(Celcius)	pH	EC (mS/m)	Dissolved oxygen (%)	Hardness (mol/L)	TDS (mg/L)	TN (ppm)	TP (ppm)	Nitrate (ppm)
A	River	6.1	6.97	0.28	83.6	2.3	24	3.072	0.193	3.81
A	Small	7.5	6.64	0.39	78.3	3.5	258	4.078	0.039	3.08
B	Small	7.8	6.55	0.24	95.2	3.35	10	0	0.055	0.01
C	River	6.1	7.16	0.16	94.2	2.8	40	1.136	0.021	1.26
C	Large	5.9	7.21	0.17	89.7	2.4	70	0.667	0.03	0.87
D	Large	5.3	7.4	0.23	75.9	1.75	20	3.625	0.001	3.79
D	Small	5.8	6.86	0.25	80.4	2.4	13	6.377	0.006	6.42
E	River	5.4	6.13	0.14	78.9	1.85	10	3.169	0.004	3.43
E	Large	5.1	6.54	0.13	75.4	2.7	20	0.082	0.002	0.54
F	River	4.9	6.68	0.11	79.3	2.95	10	2.122	0.012	2.09
F	Large	4.6	6.81	0.27	79.2	2.65	20	8.836	0.11	6.09
F	Small	4	5.79	0.11	90.1	2.3	6	2.321	0.001	2.44

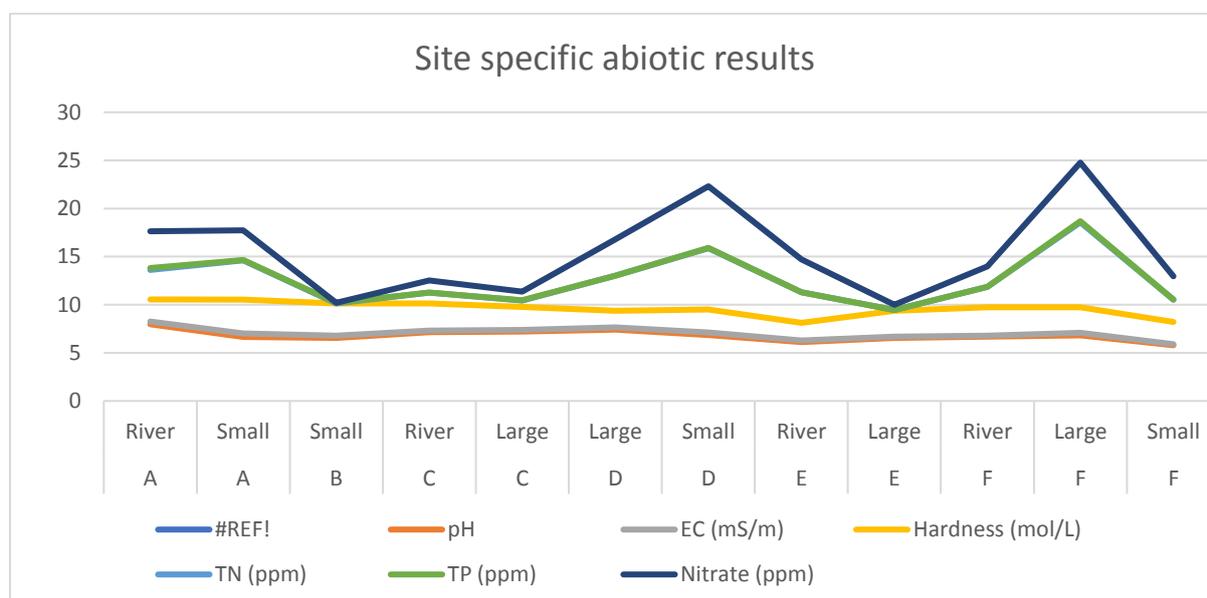


Figure 10: Site specific abiotic variables

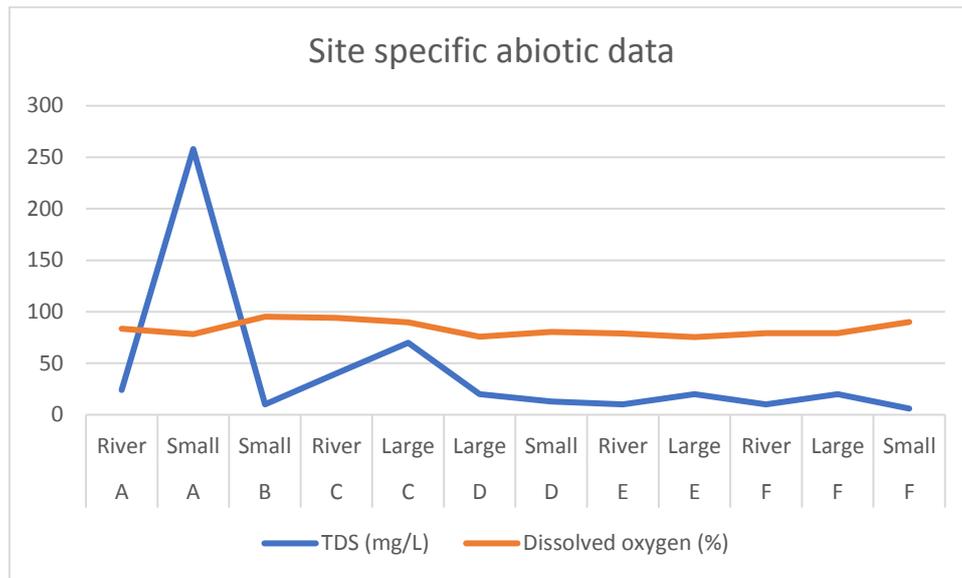


Figure 11: Site specific abiotic variables (DO and TDS)

Table 8: Site specific larvae data

Location	waterway type	Simpsons Index	Shannon Index	Species richness	Species richness ln(s)	Evenness	TOTAL # INDIVIDUALS
A	River		0	1	0	0	4
A	Small		0	1	0	0	5
B	Small	0.394	1.73	7	1.946	0.2	70
C	River		0	1	0	0	4
C	Large		0	1	0	0	3
D	Large	0.448	1.13	4	1.386	0.32	43
D	Small	0.287	2	6	1.792	0.16	119
E	River		0	1	0	0	5
E	Large	0.246	2.02	5	1.609	0.15	19
F	River	0.1	1.92	4	1.386	0.07	5
F	Large	0.466	1.21	3	1.099	0.42	35
F	Small	0.33	1.55	3	1.099	0.3	39

Table 9: Site specific biotic and abiotic factors

Location	waterway type	Aquatic vegetation (%)	Bottom type	Water colour
A	River	1	mud	Cloudy brown
A	Small	2	mud	Murky brown
B	Small	95	Mud, Organic matter	slightly brown
C	River	1	rock, silt	Clear- cloudy brown
C	Large	25	mud	Very murky brown
D	Large	8	Mud, rock	Clear, brown tinge
D	Small	90	Mud	Clear brown tinge
E	River	2	sand, rock, silt	Clear brown
E	Large	10	mud organic matter	Dark brown
F	River	50	Mud stone	Brown clear
F	Large	30	Mud, rocks	Murkey brown
F	Small	85	large stones, mud	Clear

### Appendix 5: Predators in the small pond waterbodies

It has generally been assumed that wetlands may offer a better breeding ground for Aquatic insects, including Odonata, as they lack larger predators, such as fish, however several fish were caught in 2 locations, these locations did have lower species richness and total abundance than those locations without fish, with only the common *Coenagrion* being located, highlighting this species strength and adaptivity. (images from Sara Bischoff)

Location C: Large wetland

(3 *Coenagrion* were located here)



Location A: Small pond.

(5 *Coenagrion* were located here)



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