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Marceau Minot

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# THÈSE

Pour obtenir le diplôme de doctorat

Spécialité : Écologie

Préparée au sein de l'Université de Rouen Normandie

**Biological traits and environmental factors shaping local movements and dispersal of dragonflies (Insecta, Odonata) in pond networks.**

Présentée et soutenue par  
**Marceau MINOT**

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## ***Foreword***

This PhD thesis was conducted at the Laboratory “Étude et COmpréhension de la bioDIVERsité” (ECODIV EA 1293 / USC INRAE) at the University of Rouen – Normandie in France. Professor Michaël Aubert and Dr. Aurélie Husté were the PhD co-supervisors.

My research was supported by a grant provided by the French Ministry of Higher Education and Research through the Normandy’s Doctoral School of Integrative Biology, Health and Environment (EdN BISE 497, Rouen University). The operating budget was provided by the Seine-Normandy Water Agency through the research project CROSS (Conservation Régionale des Odonates par le Suivi des critères de Survie des populations et des initiatives de gestion).

Molecular analyses of population genetics were done on the PRESEN Plateform (FR CNRS 3730 SCALE, Normandy University, France) and the Institute of Ecology and Environmental Sciences (IEES) of Paris (UMR 7618, CNRS, Sorbonne University, France). Protein quantification assays were done at the Laboratory Glyco-MEV (EA4358, Normandy University, France).

The present manuscript is composed of a general introduction, followed by five chapters presenting the results grouped in three main parts. A general discussion and perspectives are then proposed to close the manuscript.

## ***Abstract***

During the last decades, the number of ponds decreased by more than 50 % in European countries, occasionally reaching up to 90 % in some regions. Their decline in number has led to a strong loss of connectivity between waterbodies. Yet, these small and scattered ecosystems are essential for the life cycle of a high diversity of freshwater species. Land use policies like the creation of Greenways and Blueways in France aim to improve ecological continuities to allow maintenance of existent biological populations and exchanges between them. However, the connectivity between ponds must be considered according to the dispersal abilities of freshwater species and this information often lacks to guide restoration measures. In the present work, we studied the dispersal abilities of dragonflies on several spatial scales and investigated the biological traits and environmental factors that shaped their movements.

In the first part, we evaluated the colonization of 20 ponds in Normandy by dragonflies during three years after pond restoration or pond creation. The results highlight high colonization rates during the first year and no difference in species richness was found between newly created or restored ponds. This suggests that restoration of ponds after complete drought should not always be prioritized over pond creation in management strategies. We found that generalist species were more present in the first year after pond creation or restoration, whereas the occurrence of forest specialists increased with the age of the pond. The results also highlighted that the landscape context around ponds (i.e. forest vs. open lands) had an effect on the composition of dragonfly communities. Finally, the total abundance of odonate species was related to the density of other ponds in the surroundings. This result emphasizes that highly connected ponds can support larger populations than isolated ones and thus, be more resilient to perturbations.

The second part provides insights into the larval development of *Anax imperator* and the relationship between morphological traits of larvae and adults. The results suggest that the survival of this species might depend on its body length during the maturation period. We also tried to study the natal dispersal by marking 87 individuals at emergence, but only two males were resighted after the maturation period. Finally, the effect of two water pollutants (i.e. Round-up and DEET) at different concentrations was also investigated on the larval development and adults of *Aeshna cyanea*. Larvae were reared under laboratory conditions and exposed to concentrations up to 30 mg.L<sup>-1</sup> of the two pollutants. No effect of the pollutants was detected on the morphological conditions of larvae or teneral, suggesting that *A. cyanea* is tolerant to potential water pollution of ponds. The level of HSP70

stress protein was also similar according to the different treatments, but teneral adults presented higher levels of stress than larvae, suggesting that emergence induced a high stress in the individuals.

The third part focuses on the dispersal of *A. imperator*. We first assessed the local movements within a pond network in the Normandy region. Several movements between ponds were recorded, showing that individuals were able to use several ponds during their lifetime. Especially, we found that females used a larger home range than males and were more mobile in the terrestrial surroundings of ponds. We also highlighted the importance of trees used as resting sites in the vicinity of the ponds. The genetic structure of *A. imperator* populations was also investigated at both the regional and the European scales. Results indicate a high gene flow between populations in Normandy, confirming the high movement rates of *A. imperator* at the regional scale. No isolation by distance was found at the European scale. However, a genetic structure was found and Bayesian clustering analyses showed three distinct clusters (i.e. UK, France, eastern European countries). Results suggest that the English Channel may act as a barrier to gene flow.

Overall, this study provides quantifications of dragonfly dispersal abilities and insights into the biological traits and factors that could influence them at different scales. It also provides information on terrestrial habitat use by dragonflies. We finally give some recommendations for management policies to better sustain dragonfly populations in pond networks.

**Keywords:** Capture-Mark-Recapture, Connectivity, Dispersal, Dragonflies, Morphological traits, Odonates, Ponds.

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# Table of contents

<b>Foreword</b> .....	<b>i</b>
<b>Abstract</b> .....	<b>ii</b>
<b>Acknowledgements</b> .....	<b>iv</b>
<b>General introduction</b> .....	<b>1</b>
1. Study framework .....	2
1.1. A global biodiversity loss driven by anthropic activities.....	2
1.2. Assembly rules and metapopulation theory: two complementary approaches.....	6
1.3. Landscape fragmentation: a real threat for populations? .....	9
1.4. The importance of connectivity for population maintenance .....	12
1.5. Space use and dispersal: responses of the species to the landscape composition ..	14
2. Focus on pond ecosystems: biodiversity islands in anthropic landscapes.....	17
2.1. What is a pond?.....	17
2.2. Ecological function of ponds .....	17
2.3. Ponds as biodiversity hotspots .....	18
2.4. Pond loss and management initiatives .....	20
2.5. Maintaining connectivity between these scattered ecosystems .....	21
3. Dragonflies: A model for dispersal studies .....	22
3.1. Evolution of odonates and current diversity .....	22
3.2. A life cycle between aquatic and terrestrial habitats .....	24
3.3. Morphological traits.....	26
3.4. Ecology of dragonflies .....	30
3.5. Dragonfly dispersal .....	33
<b>Objectives and main hypotheses</b> .....	<b>39</b>

## PART I – Colonisation of new ponds by dragonflies

<b>Chapter 1 - Pond creation and restoration: patterns of odonate colonisation and community dynamics .....</b>	<b>45</b>
1. Introduction .....	46
2. Material and methods .....	49
2.1. Study sites .....	49
2.2. Sampling method.....	50
2.3. Environmental variables of ponds.....	50
2.4. Computation of community parameters .....	51
2.5. Data analysis .....	52
3. Results.....	54
3.1. Description of the samples .....	54
3.2. Colonisation and community dynamics .....	56
3.3. Abundance-occupancy relationships and community composition .....	58
4. Discussion.....	62
5. Acknowledgements .....	67
6. Supplementary material.....	68

## PART II - Larval development, consequences on adult condition, and natal dispersal

### Chapter 2 - Biometry of the large dragonfly *Anax imperator* (Odonata, Aeshnidae): A study of traits from larval development to adults ..... 71

1. Introduction .....	72
2. Material and methods .....	74
2.1. Sampling ponds and methods .....	74
2.2. Larvae rearing .....	76
2.3. Trait measurements of larvae and teneral.....	77
2.4. Mature adult trait measurements .....	77
2.5. Data analyses.....	77
3. Results.....	79
3.1. Larval stages .....	79
3.2. Adult traits .....	82
4. Discussion.....	86
5. Acknowledgements .....	89
6. Supplementary materials .....	90

### Chapter 3 - Effects of water pollution on the larval development and condition of the adults at emergence in *Aeshna cyanea* (Odonata: Aeshnidae)..... 93

1. Introduction .....	94
2. Material and methods .....	96
2.1. Study species and water pollutants.....	96
2.2. Larvae rearing .....	97
2.3. Trait measurements and instar identification .....	98
2.4. Experimental setup .....	99
2.5. Stress protein assay .....	100
2.6. Data analysis .....	101
3. Results.....	102
4. Discussion.....	105
5. Acknowledgements .....	107

## PART III – Dispersal of the large dragonfly *Anax imperator*

<b>Chapter 4 - Habitat use and movements of a large dragonfly (Odonata: <i>Anax imperator</i>) in a pond network.....</b>	<b>111</b>
1. Introduction .....	113
2. Material and methods .....	115
2.1. Study area.....	115
2.2. Study species .....	115
2.3. Marking protocol .....	117
2.4. Survey design .....	118
2.5. Traits measurements .....	119
2.6. Data analysis .....	119
3. Results.....	123
3.1. Survival and movements between ponds .....	123
3.2. <i>Anax imperator</i> 's home range.....	126
3.3. Habitat selection and behaviour of <i>Anax imperator</i> .....	127
4. Discussion.....	130
5. Acknowledgements .....	135
6. Supplementary materials .....	136
<b>Chapter 5 - Diversity and genetic structure of <i>Anax imperator</i> populations at the European scale.....</b>	<b>139</b>
1. Introduction .....	140
2. Material and methods .....	143
2.1. Study populations and sample collection .....	143
2.2. DNA extraction and microsatellite genotyping .....	144
2.3. Genetic diversity .....	145
2.4. Population genetic analyses and geographic structure .....	146
2.5. Migration rates between studied populations .....	148
3. Results.....	148
3.1. Genetic diversity .....	148
3.2. Population genetic differentiation .....	149
3.3. Spatial genetic structure .....	153
3.4. Recent migration rates among populations.....	157
4. Discussion.....	158
5. Acknowledgements .....	161

<b>Discussion and perspectives .....</b>	<b>161</b>
Discussion on part I: Colonisation of new ponds by dragonflies .....	162
Summary of the results .....	162
Methodological comments on the identification of larvae .....	163
Species ecological niches .....	163
Comparison between larval and adult inventories .....	165
Discussion on part II: Larval development, consequences on adult condition and natal dispersal	167
Complementary study on natal dispersal.....	167
Summary of the results .....	172
Methodological comments on harmonic radars.....	173
Discussion on natal dispersal .....	174
Discussion on part III: Dispersal of the large dragonfly <i>Anax imperator</i> .....	176
Summary of the results .....	176
Methodological comments on the marking methods .....	177
From local movements to dispersal during the mature adult life: a female biased dispersal? ...	179
General discussion and perspectives .....	181
Linking life traits to dispersal .....	181
Connectivity and management of pond networks in Normandy.....	182
Further challenges and perspectives for dispersal studies on dragonflies.....	184
Appendix: Criteria used for the identification of 23 dragonfly species found at larval stage in ponds of Normandy .....	187
<b>References.....</b>	<b>191</b>

# General introduction

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The focus of this thesis work was to study the movements of dragonflies within pond networks in Normandy. Our approach is based on several ecological concepts, from ecological factors that condition species occurrence and population maintenance, to landscape ecology that shapes dispersal movements between ponds. In this general introduction, we first present the main threats to global biodiversity and we develop the ecological concepts explaining species assemblages and population dynamics. We then present the importance of connectivity for population persistence and how landscape factors can shape local movements and dispersal events. This study framework is essential to understand the ecology of our study model “ponds” and study species “dragonflies” that are developed in the second and the third part of this general introduction.

# 1. Study framework

## 1.1. A global biodiversity loss driven by anthropic activities

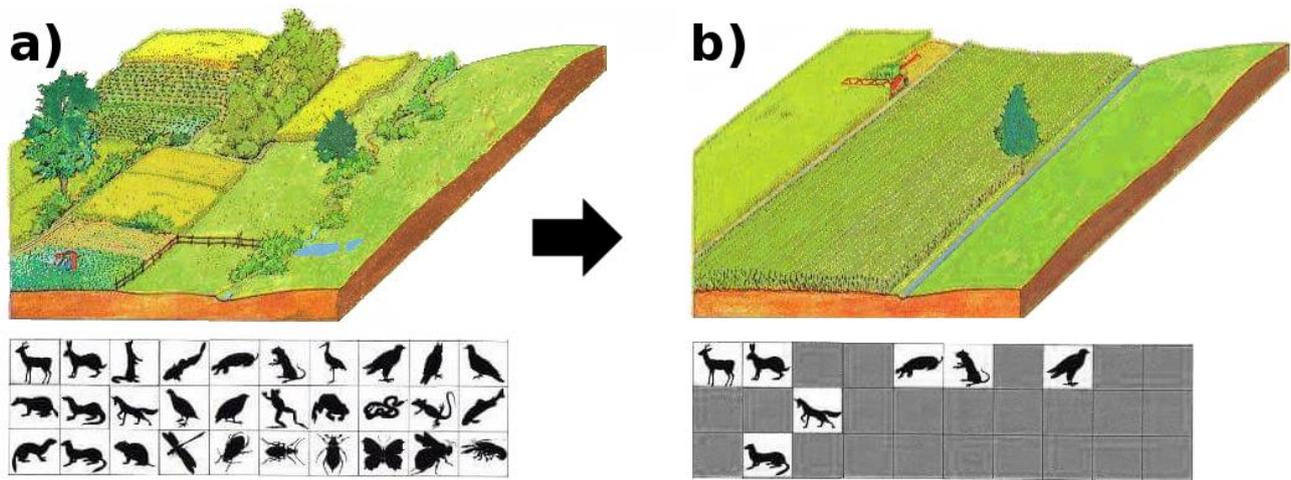
The rapid growth in human population since the middle of the 20th century and associated anthropic activities have a dramatic impact on biodiversity all over the world (Pereira et al. 2012). The main threat on global biodiversity is overexploitation, especially logging and deforestation in tropical regions, often associated with agricultural expansion (Maxwell 2016). In Europe, the forest area is not decreasing (European environment agency (EEA) 2018), but biodiversity still suffers from a strong decline due to anthropic activities, especially changes in agricultural practices and urban development (EEA 2015a).

### *Land use change in Europe*

Forests and other wooded lands represent about 40 % of the European Union area with a slight increase during the last decades (EEA 2015b). These ecosystems are mildly anthropized but most of the forests are harvested for wood supply. The management practices especially clearcuts and changes in tree composition can affect species in terms of abundance and community structure (*e.g.* Paillet et al. 2010; Quine & Humphrey 2010; Henneron et al. 2018). However, despite these potential threats on biodiversity, little or no decline of European forest plants or insect species was reported over the last decades (Quine & Humphrey 2010; Schulze 2018).

Agricultural surfaces also cover about 40 % of the European area (EEA 2016). The rapid evolution of practices at the end of the last century has led to a significant increase in agricultural yields thanks to land consolidation, intensive use of fertilizer inputs and biological control agents. This intensification of agriculture led to the disappearance of many habitats (*e.g.* hedges removal, wetland draining) and the simplification of the initially heterogeneous landscapes to homogeneous areas (Fig. 1; Husson et Marochini 1997). As a consequence, many rural taxa (*e.g.* birds, mammals, arthropods, flowering plants) suffered from a drastic decline in species richness and species abundance (Benton et al. 2003; Hendrickx et al. 2007; Bowler et al. 2019).

Moreover, urban sprawl and intensification of the connections between cities add further pressures on the landscapes by draining or sealing surfaces. In Europe, the amount of artificial surfaces represents 5 % of the European area (EEA 2016) and increased by more than 750 km<sup>2</sup> per year (*i.e.* + 6.7 %) during the past two decades (EEA 2019a). This changes in land use are responsible for a decrease in the surface of agricultural lands (*i.e.* 78 % were taken from agricultural areas). They may also have a strong effect on landscape by fragmenting large habitat patches into smaller ones (EEA 2019).



**Fig 1.** Example of agricultural landscape and associated biodiversity (a) before and (b) after land consolidation (adapted from Schuck 2018).

### Pollution

Pesticides and herbicides for domestic and agricultural uses are, by definition, designed to eliminate many target plants and insect ‘pests’. However, they are also responsible for the disappearance of non-target species, especially arthropods, with consequences on the whole food chain (Sánchez-Bayo 2011; Dudley et al. 2017). Soil fertilisation with nitrogen and phosphorus inputs significantly increases the productivity of crops and ornamental flowers. However, their intensive use results in the eutrophication of aquatic environments and nearby terrestrial ecosystems via water run-off<sup>1</sup> (Piney et al. 2018). In aquatic environments such as ponds, eutrophication leads to the rapid growth of macrophytes like *Lemna* sp. that rapidly colonise the whole water surface, preventing the light from penetrating in depth and thus, decreasing the oxygenation of the water body with consequences on the entire food web (Jenderedjian et al. 2012; Piney et al. 2018). In terrestrial environments, oligotrophic plants also tend to be replaced by neutrophilic species leading to an overall decline in plant diversity and associated insects (Öckinger et al. 2006; Payne et al. 2017). Other pollutants like heavy metals released by road traffic (Visioli et al. 2013) or occasional industrial spills (Giger et al. 2009) may also have local effects on biodiversity. Even at sublethal concentrations, the presence of pesticides in aquatic ecosystems can affect the development of aquatic invertebrates (Tüzün & Stoks 2017). However, their impact on global biodiversity is complex to assess because they often interact with other environmental factors like temperature (Stephansen et al. 2016; Verheyen & Stoks 2020).

<sup>1</sup> water run-off: excess of water that cannot infiltrate the soil

### *Invasive alien species*

The intensification of daily traffic between continents has led to the displacement of many plant and animal species (van Kleunen et al. 2015). Few introductions occur intentionally (*e.g.* food resource, pets, biological control), but most of them remain accidental (Roques et al. 2016). Invasive Alien Species (IAS) are species “which are introduced outside their natural past or present distribution area and succeed in surviving and subsequently reproducing” and “whose introduction and/or spread threaten biological diversity” (Commission of the European communities 2008). Cities are the main entries for these species that are often very competitive in urban or degraded environments, before their spread to neighbouring ecosystems (McNeely 2001). There are many different processes by which IAS lead to the decline of native species (Ricciardi et al. 2013). For instance, mammalian predators like cats, rats or stoats threaten native species through predation, competition for resources and disease transmission (Doherty et al. 2016). At some places, invasive plants can also replace native vegetation, which often induce a decline of overall animal abundance, diversity and fitness (Schrimel et al. 2015). Finally, some species like invasive crayfish are responsible for damages to native habitat by destroying vegetation and increasing water turbidity (Carreira et al. 2014). The propensity of IAS to develop quickly in disturbed ecosystems often complicate the efforts of the managers in habitat restoration (Buckley et al. 2007).

### *Climate change*

The current climate warming trend has an increasing impact on the world’s ecosystems (IPCC 2013). In temperate regions, warming might have a positive effect on the fitness of some ectotherm populations (*e.g.* insects, frogs, turtles, lizards), by increasing their thermal performances (Deutsch et al. 2008). On the contrary, species that have low tolerance to thermic variations will suffer from this increase in temperature, and will need either to acclimate, adapt or disperse (Walther et al. 2002; Williams et al. 2008a). A shift in distribution range toward the poles (ca. 17 km per decade) and/or higher altitudes (ca. 11 m per decade) has already been reported for most terrestrial taxonomic groups (Hickling et al. 2006; Chen et al. 2011; Pecl et al. 2017). However, individual movements may be constrained by biogeographical barriers to dispersal like seas (Atterby et al. 2010) or mountains (Razgour et al. 2014), and the lack of favourable habitats (Peterson et al. 2002). These populations suffer more and more from severe decline in abundance or extinction (Dirnböck et al. 2011; Kobiv 2017). Climate change also results in a higher frequency of extreme events like drought that will, among others, increase the frequency of fires (Kasischke & Turetsky 2006) and impair the functioning of temporary ponds (Zacharias & Zamparas 2010).

The response of biodiversity to land use change, pollution, biological invasions and climate change is variable among geographical areas and taxa. For instance, birds with small distribution ranges or endemic to islands are more affected by invasive species, whereas mainland birds suffer essentially from habitat loss (Clavero et al. 2009). The effect of these threats to biodiversity may also be locally mitigated by biotic interactions. In Asian farm ponds, the impact of the invasive bullfrog on the native wrinkled frog also depends on the presence of common carps that prefer feeding on bullfrog tadpoles than on wrinkled frog tadpoles (Atobe et al. 2014). These threats to biodiversity can interact together leading to a higher global impact. For instance, climate change may reduce the adaptive capacity of native species and thus, compromise their chances to compete against generalist invasive species (Mainka & Howard 2010). Habitat loss due to artificialization and agricultural practices also often facilitate the colonisation by invasive species that are more competitive in disturbed areas (Didham et al. 2007). Overall, the biodiversity suffered from a strong decline in Europe over the last decades (*e.g.* 35% loss in grassland butterflies and 32% loss in farmland bird populations between 1995 and 2020; EEA 2019b). This decline occurs even in protected areas that are less concerned by land use change, pollution or invasive species. For instance, in 63 nature reserves from Germany, the total biomass of insects collected in traps has decreased by more than 75% in 27 years (Hallmann et al. 2017). Such a loss emphasizes the fact that even ecosystems protected from anthropogenic pressures are also affected by the global population decline of surrounding lands (Hallmann et al. 2017). Besides this decline in species richness and population size, anthropogenic pressures also lead to biotic homogenization (Olden & Rooney 2006). For instance, in Canada, human activities are responsible for a loss in rare butterfly species associated with an expansion of widespread generalist species. This change in species composition tends to shift initially diverse species pools into homogeneous assemblages of species across the country (White & Kerr 2007).

## 1.2. Assembly rules and metapopulation theory: two complementary approaches

The effective population size and its distribution (*i.e.* extent of occurrence<sup>2</sup> or area of occupancy<sup>3</sup>) are the two main components used to evaluate the level of threat to a given species (IUCN 2012). The effective population size relies on reproductive individuals only because juvenile or sterile individuals are not able to perpetuate the species. Distribution and abundance of populations usually present a positive relationship that remains consistent in time. Populations that have a low abundance often present a smaller occupancy area and fewer occurrences (Gaston et al. 2000). Understanding mechanisms that drive species occurrence, abundance and resulting species assemblages is determinant to tackle the ongoing global biodiversity crisis (Gaston 1999; Martinez et al. 2017).

### *Assembly rules: the filtering framework*

Assembly rules provide a framework based on several levels of filters, also called “filtering framework”, to explain the occurrence of a given species at a given site and the resulting species assemblages (Keddy 1992; Götzenberger 2012; Münkemüller et al. 2020). These rules describe five species pools corresponding to different levels of abiotic and biotic filters that work in a hierarchically nested pattern along both spatial and temporal scale gradients (Belyea & Lancaster 1999; Aronson et al. 2016). The initial pool of species, occurring on a large scale is called (1) the biogeographical pool. This pool results from long historical and evolutionary processes of species that occupy the same biogeographical region (Carstensen et al. 2013). The geographical distance and or the physical barriers to dispersal (*e.g.* sea, mountains) between regions act as a first filter that restricts the colonisation capacities of some species, leading to the formation of (2) geographic species pools (Morand et al. 2000). The different habitat requirements of species from a given geographic pool, along with other environmental constraints (*e.g.* temperature, pH) act as a second filter that constitutes (3) the habitat pool of species (Belyea & Lancaster 1999). The interaction between geographical constraints and environmental conditions forms a last abiotic filter to constitute (4) the ecological pool of species (Belyea & Lancaster 1999). This pool of species is then constraint at a local scale by a biotic filter comprising inter- and intra-specific interactions that may either facilitate (*e.g.* mutualism, commensalism) or limit (*e.g.* competition, predation) the occurrence of a given species (Kelt et al. 1995; Bruno et al. 2003; Lortie et al. 2004). The remaining species that were able to pass through all the filters constitute (5) the actual pool of species (Fig. 2a; Belyea & Lancaster 1999).

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<sup>2</sup> extent of occurrence: area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the sites of present occurrence of a taxon (IUCN 2012)

<sup>3</sup> area of occupancy: area is occupied by a taxon excluding unsuitable or unoccupied habitats (IUCN 2012)

*Metapopulation theory*

In a single population, the abundance of individuals is dynamic over time due to stochastic processes of demography<sup>4</sup> (Méndez et al. 2019). The probability of extinction of a population increases with the intensity of this demographic stochasticity and decreases with the size of the population. At low population sizes, a density dependent threshold can also occur (*i.e.* Allee effect; Stephens et al. 1999, Méndez et al. 2019) under which the population is not viable anymore and enters an extinction vortex<sup>5</sup> (Shaffer 1981; Fagan & Holmes 2006). Over a long time, most populations are predicted to go extinct due to their demographic stochasticity of abundance (Foley 1994; Ovaskainen & Meerson 2010) or extrinsic perturbations like occasional drought events (Hanski 1998; Chase 2007). Population dynamics studies originated with the MacArthur & Wilson theory (1963) that gave the first explanations of population maintenance over time. The dispersal of some individuals between patches allows “source” populations with a high abundance to colonise empty patches or rescue populations with lower abundance called “sinks” (Pulliam 1988). In pond networks, some waterbodies can be colonised by adult dragonflies but the seasonal droughts might prevent the larval development acting therefore as sink for populations (McCauley et al. 2008a; Suhonen et al. 2014). The asynchronous dynamic of local extinctions and re-colonisations between populations create an equilibrium that enable the persistence of a meta-population over time (Levins 1969; Hanski et al. 1996; Fig. 2b). In freshwater ecosystems, even populations of very small waterbodies often play a key role of rescue effect for nearby larger population (Semlitsch et al. 1998).

Besides the demographic effects of rescue and colonisation events, the movements of individuals between the populations also enable the genetic mixing among populations (Baguette et al. 2013). In small isolated populations the high degree of inbreeding leads to a loss of alleles, and consequently a loss of genetic diversity, due to a process of genetic drift (Allendorf 1986). These populations may become less resilient to environmental changes and more prone to extinction (Christie & Knowles 2015). On the contrary, gene flow between populations can favour local adaptations to environmental conditions (Jacob et al. 2017). In the current context of climate change, the capacity of species to adapt to changing local environmental conditions will become increasingly important for the populations to persist (Tigano & Friesen 2016).

The concept of metapopulations was also extended to communities, to account for multiple species linked by dispersal that can potentially interact with each other forming a meta-community (Wilson 1992; Leibold 2004). The theoretical frameworks of meta-populations and meta-communities

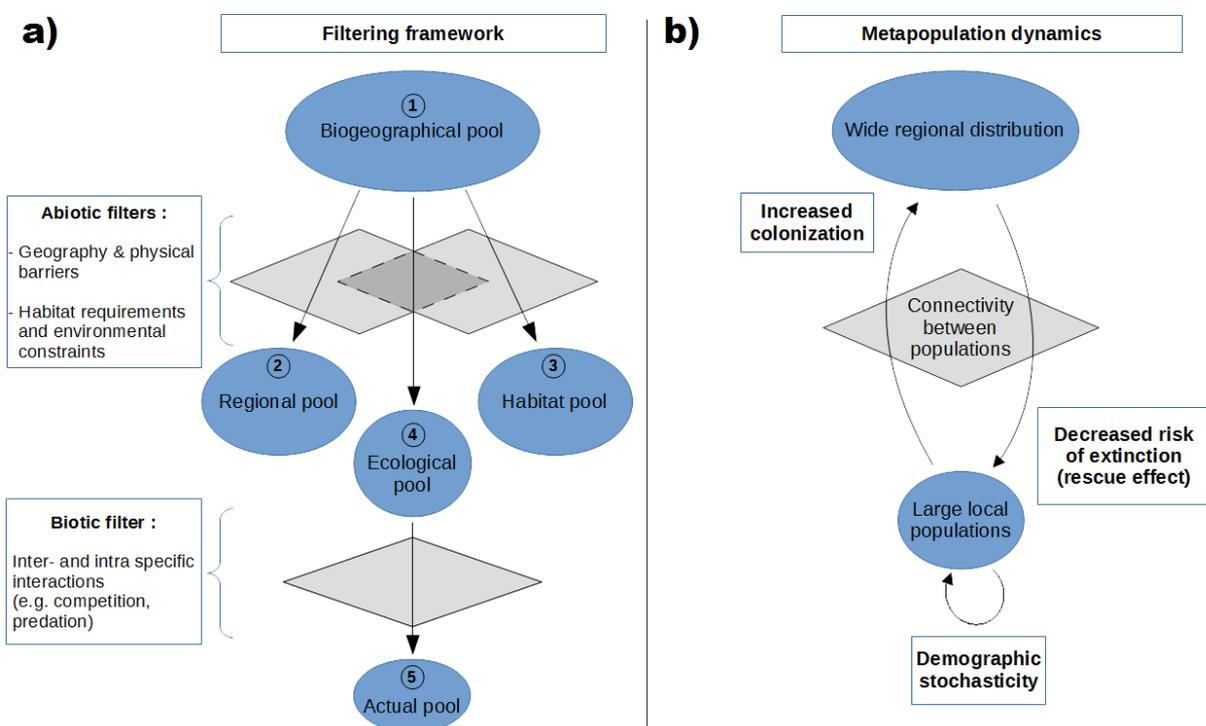
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<sup>4</sup> demographic stochasticity: random fluctuations in population size that occur because the birth and death of each individual is a discrete and probabilistic event (Melbourne 2012)

<sup>5</sup> extinction vortex: insidious mutual reinforcement that can occur as populations decline (Fagan & Holmes 2005)

provide a basis for population viability analysis (PVA) and drive conservation measures to prevent species extinction (Ovaskainen & Meerson 2010; Fox et al. 2017; Howell et al. 2020).

Overall, these approaches of both the filtering framework and the metapopulation theory are complementary. For instance, in freshwater ecosystems, the factors driving species composition can be very different depending on the size and isolation of the considered waterbodies. In large lakes supporting large populations, the species assemblages are mostly shaped by environmental factors, whereas dispersal abilities are often the limiting factor that shapes species assemblage in small and isolated ponds (Heino et al. 2015).

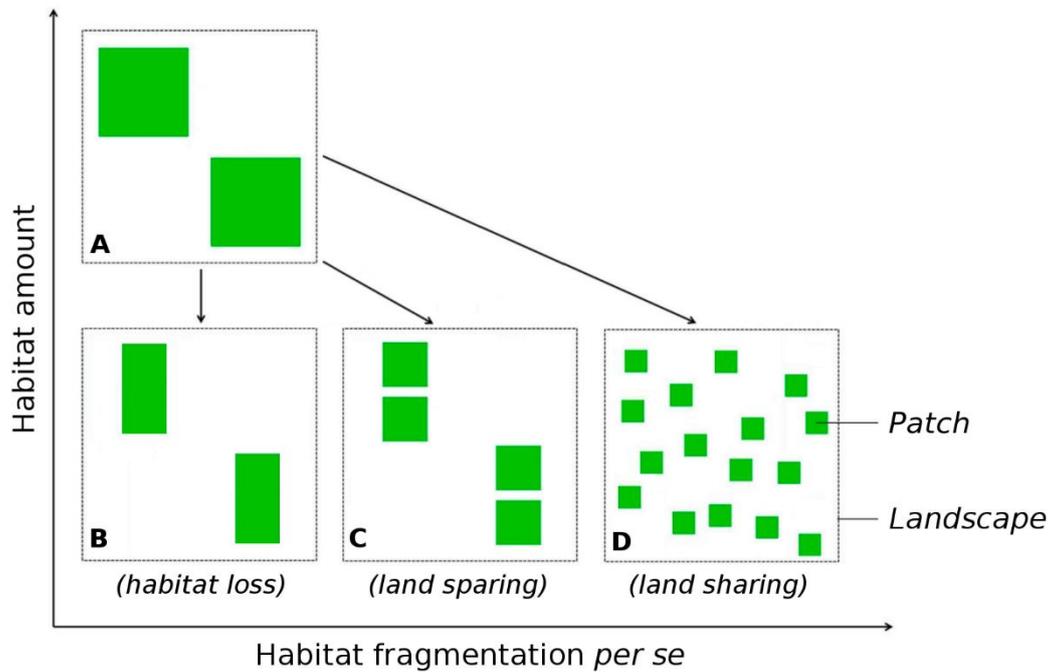


**Fig 2.** Theoretical representation of the filtering framework adapted from Belyea & Lancaster 1999 (a), and theoretical representation of the metapopulation theory adapted from Verberk et al. 2010 (b).

### 1.3. Landscape fragmentation: a real threat for populations?

Landscape fragmentation is a process by which human activities transform “*large habitat patches into smaller, more isolated fragments of habitat*” (Jochen et al. 2016). At the European scale, the number of the fragments per km<sup>2</sup> increased by 3.7% between 2009 and 2015 (EEA 2019).

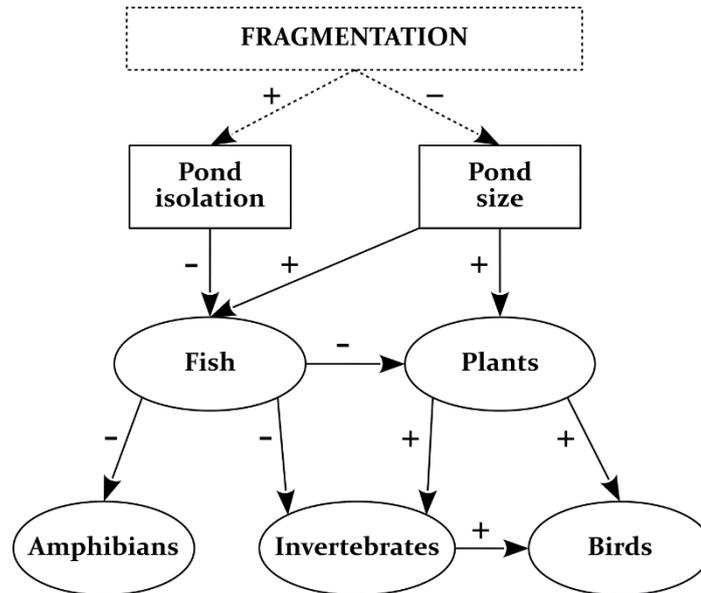
The level of fragmentation in a landscape is a crucial driver of metapopulation dynamics because the spatial configuration and the nature of the remaining habitat patches conditions the rate of exchanges between populations (Cooper et al. 2012). The process of habitat fragmentation is often associated with habitat loss and the effects of both are hard to disentangle (Didham et al. 2012; Liu et al. 2016). Moreover, in most studies on the effect of habitat fragmentation, the loss of habitat has pervasive and negative effects that probably overwhelm the effect of the fragmentation itself (Fahrig 2003, 2013; De Camargo et al. 2018). Studies on the mechanisms of fragmentation, such as the reduction of patch area, isolation and edge effect, and their dynamic in time, support the idea that fragmentation has also a negative effect on the diversity of habitat patches (Haddad et al. 2015). In forests for instance, fragmentation increases the number of habitat edges, in which large and old trees are replaced by pioneer species, leading to a negative impact on insect community composition (Haddad et al. 2015). Increasing edges in the landscape was also shown to increase the risk of negative pressures such as livestock incursions, wildfires, logging or human-wildlife conflicts and favour essentially generalists and invasive species (Fletcher et al. 2018). Nevertheless, the presumed negative effect of fragmentation “*per se*” (Haila & Hanski 1984) is still debated within the scientific community (Cooper et al. 2012, Fletcher et al. 2018, Fahrig et al. 2019). Fragmentation *per se* consists in an augmentation of the number of patches keeping the same amount of available habitats in the landscape (Fahrig 2017; Fahrig et al. 2019; Fig. 3).



**Fig 3.** Illustration of habitat loss and fragmentation processes at the landscape scale. Habitat loss (from A to B) is the process whereby habitat patches are destroyed over time. It is often associated with fragmentation, leading to land sparing (A to C) or land sharing (A to D). In contrast fragmentation *per se* only describes the difference in spatial pattern (between B, C and D) without any habitat loss (based on Fahrig 2017 and Fahrig et al. 2019).

A recent meta-analysis on fragmentation *per se* in landscapes showed that most effects of fragmentation on biodiversity were non-significant, and that significant effects can be more often positive than negative (Fahrig et al. 2017). These significant effects included changes in measured abundance, species richness or movements success. Even specialist and rare species that often tolerate a low level of disturbance did not respond negatively to habitat fragmentation *per se*. Significant positive effects of fragmentation *per se* on biodiversity were explained by: (1) a better functional connectivity of the landscape, because increasing the number of smaller patches reduces the distance between them; (2) a higher diversity of habitats because several small patches are more likely to capture a large heterogeneity than a single large patch; (3) the increased amount of edges that are more productive with higher food availability and more structurally diverse providing refuge from predation; (4) a stabilization or increased persistence of predator-prey and host-parasite interactions by allowing for instance the prey to stay a step ahead of the predator; (5) a lower inter- and intraspecific competition due to higher number of available territories, with edges of patches acting as territory boundaries; (6) spreading the risk of extinction of the species on several local interconnected populations; (7) a better landscape completion, by increasing the accessibility of different habitats. Therefore, creating many small patches (*i.e.* land sharing) across a landscape may result in a higher

biodiversity outcome than few large patches (*i.e.* land sparing) for the same surface of habitat (Fahrig et al. 2017). An example of this counter-intuitive increase in biodiversity in smaller patches was described in shallow lakes and ponds due to second order effect of species interactions with fish (Fig. 4; Scheffer et al. 2006). This change of paradigm about the effects of fragmentation are therefore expected to bring new insights for biodiversity conservation in the management of landscapes like agroecosystems or wetlands (Dudley et al. 1997; Semlitsch & Bodie 1998).



**Fig. 4.** Representation of pathways through which fragmentation of habitat patches (here ponds) can affect different groups of aquatic organisms (adapted from Scheffer et al. 2006).

Overall, the effects of fragmentation on biodiversity are variable and the response of a given species probably depends on the scale of the study (De Camargo 2018; Fahrig, 2019). While fragmentation seems to have a negative impact at the patch scale (Fletcher et al. 2018), the mosaic of patches obtained by fragmentation *per se* at a larger scale (*i.e.* landscape scale) may also enhance biodiversity (Hovick et al. 2015; Erdős et al. 2018). Although there is no scientific consensus on the impact of fragmentation on biodiversity, all agree that landscape connectivity is essential to avoid habitat isolation (Fletcher et al. 2018; Fahrig et al. 2019). Patch isolation occurs when a habitat is too far or physically separated from similar patches, such that inhabiting species are unable to reach another patch (Ricketts 2001). In small patches hosting a limited number of individuals, isolation leads to inbreeding and genetic drifts in the populations (Ellegren & Galtier 2016). These populations become less resilient and are more vulnerable to local extinction (Griffiths et al. 2020). Therefore, restoration measures in favour of biodiversity are inefficient if they are only applied on isolated habitats.

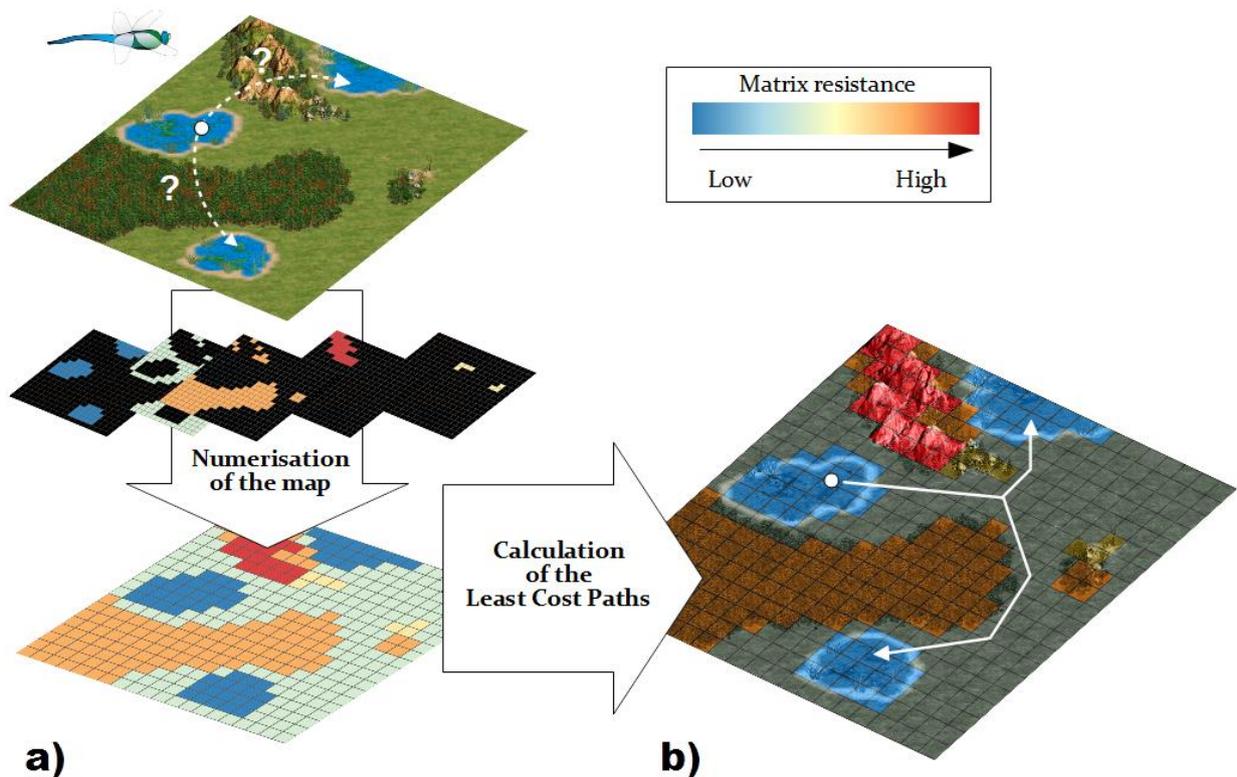
## 1.4 The importance of connectivity for population maintenance

In this context of habitat loss and fragmentation, European countries signed in 1995 a Pan-European Biological and Landscape Diversity Strategy that aimed to stop global biodiversity loss by 2020. To achieve these objectives, French land use policies started a Greenway and Blueway (*i.e.* 'Trame Verte et Bleue', TVB) initiated by the Grenelle de l'Environnement held in Paris in 2007. This TVB is essentially a legislative system driving urban planning documents from regional to city scale. These documents must specify ecological continuities (*i.e.* green and blue ways) on the territory to allow maintenance of existent populations and exchanges between them. For example, linear corridors of large trees may be planted in urban areas to allow the maintenance of bird movements (Matsuba et al. 2016). Waterway continuum also can be re-established by removing unused dams and weirs or installing fish passages to allow fish migration along the streams (Watson et al. 2018). Fauna passages may also be created to mitigate the adverse effects of new roads on amphibians and small mammal populations (Mata et al. 2008).

Connectivity may either be studied at the landscape scale or at the patch scale (Spanowicz & Jaeger 2019). Landscape connectivity can be defined as "*the extent to which a landscape facilitates the movements of organisms and their genes*", whereas patch connectivity only focuses on movements between discrete habitat patches without considering within patch movements (Rudnick et al. 2012). A good landscape connectivity promotes long distance movements as well as local movements of species on different spatio-temporal scales (Balbi et al. 2019). Connectivity can be divided into two major dimensions: structure and function. Structural connectivity describes the physical characteristics of the landscape (*e.g.* topography, land cover or hydrology) and does not depend on the dispersal abilities or behaviour of organisms. Functional connectivity describes behavioural responses of organisms to landscape elements (Kindlmann & Burel 2008).

Several methods can be used to measure the structural connectivity. Simple measurements of connectivity may be performed by calculating the Euclidian distance to the nearest suitable patch or the percentage of habitat cover in a buffer zone. More complex metrics were also developed such as the Connectance Index (McGarigal et al. 2002) or the integral index of connectivity (Pascual-Hortal & Saura 2006). These metrics permit for instance to test the structural importance of an individual pond to the entire network connectivity and predict the potential consequences of a pond loss (Ribeiro et al. 2011). However, the connectivity of a landscape can also be assessed by using models at a larger scale such as the Least-Cost Path (LCP) analysis (Balbi et al. 2019). These models are more and more developed as the computational power and availability of large datasets increase. LCP models are based on the principle that, to move between habitat patches, organisms need to cross a part of the

landscape called “matrix” (Kindlmann & Burel 2008). Depending on its composition and on the requirement of the studied species, the landscape matrix can be more or less permeable to movements. For instance, some dragonfly species (*e.g. Leucorrhinia intacta*) are more likely to undertake movements in open fields than in forests, due to their structural complexity, lower light availability, and temperature (French & McCauley 2019). A grid map of the landscape is created and each unit is assigned a value of resistance to movement based on available data of habitat preferences for a given species (Fig. 5a; Adriaensen et al. 2003). The LCP analysis then consists in calculating the best path to connect different sites on the map by minimizing the cumulative resistance to movements of the matrix (Fig. 5b; Etherington 2019; Balbi et al. 2019). Identifying the most likely route between target populations or habitat patches enables then to direct protection efforts and mitigate the impact of barriers (Cushman et al. 2013; Vasudev et al. 2015). Other biotic factors like the predation risk might also be combined with the matrix permeability to improve the predictions of the LCP models (Winandy et al. 2019). For the LCP to be biologically relevant, ecological corridors must be of a reasonable length, so that dispersal abilities of the studied species enable to cross it. The calibration of these models relies therefore on available datasets of movements, and requires distinguishing between local movements and dispersal movements (Blazquez-Cabera 2016).



**Fig. 5.** Theoretical Least Cost Map (a) and Least Cost Path (b) selected by a dragonfly species which is more mobile in open field landscapes than in forests or mountain regions.

## 1.5. Space use and dispersal: responses of the species to the landscape composition

During its lifetime, an animal performs several types of movements, from foraging and reproductive movements to dispersal and migration (Ims 1995; Van Dyck & Baguette 2005).

Local movements aiming to find resources and to mate are performed in a defined area named “home range” (Walton 2017). This concept was first developed in mammal life history (Seton 1909, Burt 1943) and has then been extended to other groups (Hagen et al. 2011; Valenzuela-Sánchez 2014; Wood et al. 2017). Home ranges are usually studied using datasets of animal locations obtained by Capture-Mark-Recapture (CMR) surveys, especially using telemetry methods. It can be estimated either by calculating the area covered by the most distant recorded locations (*i.e.* Minimum Convex Polygon) or based on the distribution of location points (*i.e.* Kernel Utilisation Distribution; Börger 2006). Home range size and composition constitute an important study frame because they structure species interactions, trophic processes and community composition (Walton et al. 2017). In terms of conservation, information provided by home ranges gives guidelines on the areas to be specifically protected (Schofield et al. 2010). Home range is often restricted to a specific interval of the animal’s life (*e.g.* juvenile or mature stage; Powell & Mitchell 2012). In some cases, local movements can be hard to distinguish from dispersal events because the sum of daily routine movements can lead to actual dispersal (Van Dyck & Baguette 2005).

Dispersal can be defined, in a broader sense, as the movement of an organism between different populations or habitats. These movements can either lead to a successful reproduction (*i.e.* effective dispersal) or not (Lancaster & Downes 2017). Clobert et al. (2001) distinguish two types of effective dispersal: the natal dispersal (*i.e.* “*the movement between the natal area or social group and the area or social group where breeding first takes place*”) and the breeding dispersal (*i.e.* “*the movement between two successive breeding areas or social groups*”). Individuals that disperse without reproduction are called ‘itinerants’ (Lancaster & Downes 2017). Their movements may be motivated by foraging activities (*e.g.* dragonflies in terrestrial habitat; Foster & Soluk 2006), but they may also be unable to breed in a favourable habitat (*e.g.* due to high competition, predation) or fall into ecological traps<sup>6</sup> (Robertson & Hutto 2006; Harabiš & Dolný 2012).

Dispersal abilities and underlying mechanisms that condition individual movements are very variable among species (Lowe & McPeck 2014). The propensity of an animal to disperse is mainly driven by dispersal traits (*e.g.* wing morphology) or behaviours (*e.g.* settlement location, rate of

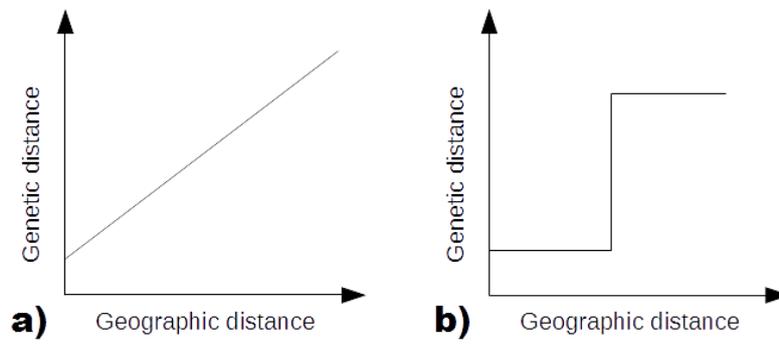
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<sup>6</sup> ecological trap: poor habitat that becomes relatively more attractive thus “baiting” individuals to settle (Robertson & Hutto 2006)

activity) that rely on a genetic basis (Saastamoinen 2017). However, dispersal is also often conditioned by external biotic and abiotic factors. Many species of insects, birds, fish or turtles rely, at least partly, on the wind or aquatic currents for long distance movements such as migration (Bowen & Karl 2007; Troast et al. 2016; Haest et al. 2019). Several animals, especially invertebrates, also rely on other organisms to disperse (Bilton et al. 2001). Their movements depend on their propensity to be carried and survive the journey, as well as on the dispersal abilities of the vector organism (Jordano 2017). Dispersal events can either cover a limited distance (Short Distance Dispersal: SDD) or a long distance (Long Distance Dispersal: LDD). The definition to distinguish between these two categories depends on both the study organism and the landscape context or habitat. LDD is characterized by a very low probability and must allow to reach extremely long distances compared to other SDD events (Nathan et al. 2003; Jordano et al. 2017). For instance, local movements of 100 m are common in most butterfly and dragonfly species (Conrad et al. 1999; Nowicki et al. 2014), but can be very rare and considered as dispersal in flightless insect species (*e.g. Chorthippus montanus*; Weyer et al. 2012).

The LDD events are very rare at the individual scale, but have significant impacts on population maintenance by favouring colonisation of new patches and gene flow (Jordano 2017). Although sometimes challenging due to the rarity of these events, the detection of SDD and LDD is generally performed using mark recapture methods, including telemetry (Bennetts et al. 2001; Curry 2018).

The dispersal of individuals from one population to another and subsequent reproduction contribute to increasing the local genetic diversity of populations (Baguette et al. 2013). Gene flow is therefore strongly associated with the dispersal process and can even be used to define the dispersal itself as: “any movement of individuals or propagules with potential consequences for gene flow across space” (Ronce 2007). The observed genetic structure of a population cannot provide an estimation of dispersal, but it enables to quantify the degree of genetic differentiation between populations. Landscape genetic studies aim to analyse the effect of the landscape on gene flow by combining methods of landscape ecology and population genetics (Storfer et al. 2010). Based on the study of gene flow, measures of genetic differentiation like  $F_{ST}$  provide indirect information on past dispersal events between populations (Weir & Cockerham 1984). The genetic distance between populations often increases with the Euclidean distance separating the studied populations following a relationship called “isolation by distance” (IBD; Fig. 6a; Rousset 1997). However, the pattern of genetic distance can also present an abrupt increase due to a geographical barrier to gene flow (Fig. 6b; Baguette et al. 2013; Ringbauer et al. 2018). Studies on population genetics constitute therefore a crucial basis to identify these barriers to dispersal and provide guidelines for conservation and connectivity restoration strategies (Kelly & Phillips 2016; Shaw et al. 2016).



**Fig. 6.** Illustration of the relationship between genetic structure and the geographical distance in case of isolation by distance (a) and with the presence of a barrier to geneflow (b).

## 2. Focus on pond ecosystems: biodiversity islands in anthropic landscapes

### 2.1. What is a pond?

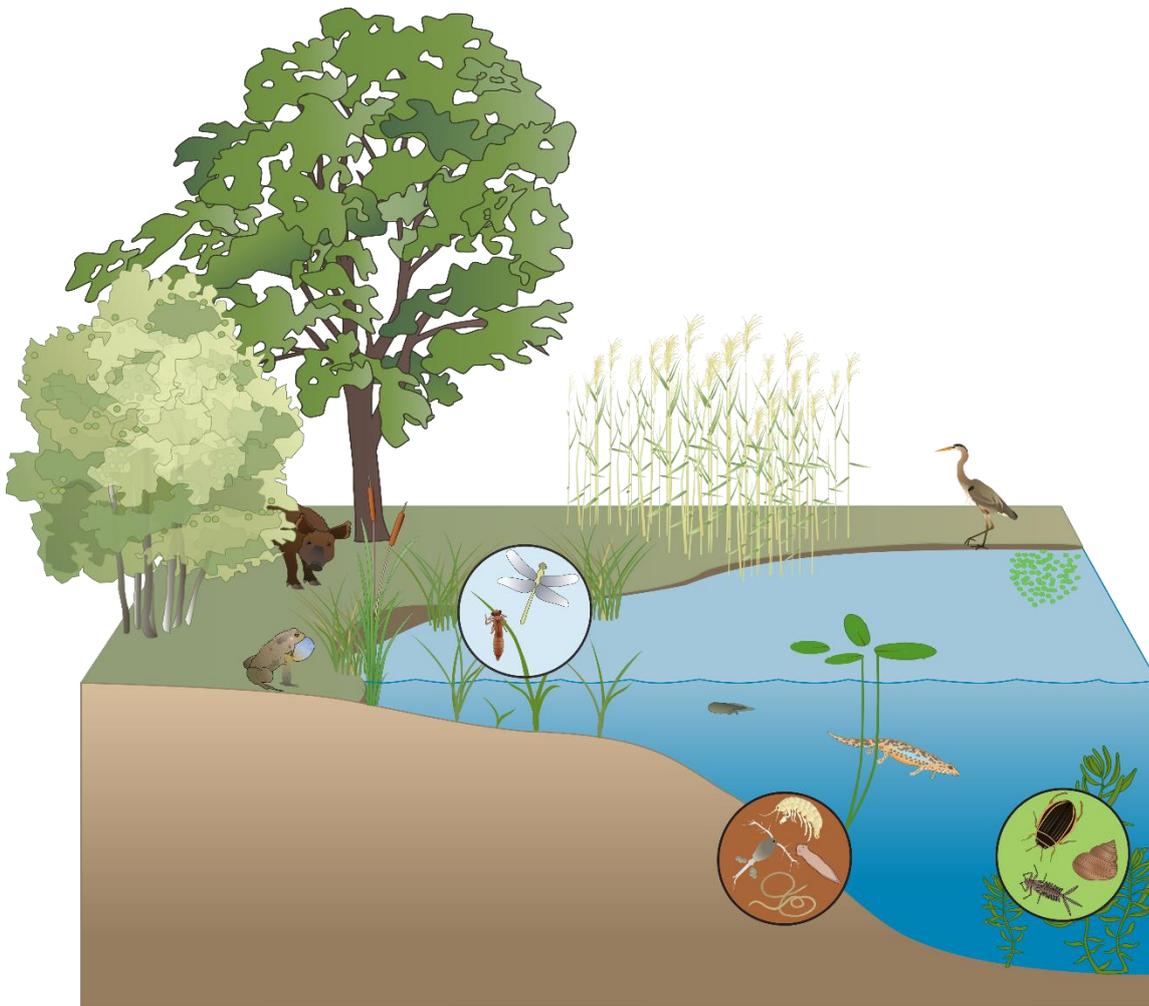
There is no clear consensus on the definition of ponds, especially regarding their size, depth and nature of management. Some may also be categorized as “wetlands” in North America (Biggs et al. 2017). The European Pond Conservation Network (EPCN) defines a pond as a waterbody “*between 1 m<sup>2</sup> and 2 ha in area which may be permanent or seasonal, including both man-made and natural waterbodies*” (Biggs et al. 2005), “*with a maximum depth of no more than 8 m, offering water plants the potential to colonise almost the entire area of the pond*” (Oertli et al. 2005). These definitions allow to separate ponds from lakes, whose depth prevents the light to reach the bottom, making it unsuitable for aquatic vegetation. Several studies also showed that a change in the relationship between species richness and pond area occurs when pond area exceeds about 2 hectares (Catalan et al. 2009; Hamerlik et al. 2014). Ponds are present in all biogeographical regions of the globe and their total surface reaches about 30 % of the lentic freshwater surfaces on earth (Céréghino et al. 2014). Recent estimates suggest that their number represent more than 90% of the standing waterbodies with ca. 500 million ponds on earth (Holgerson & Raymond 2016). The European Union included ponds in its European Water Framework Directive (WFD) adopted in 2000 (European Commission 2000). Following this dynamic, France also took ponds into account in the TVB scheme (Allag-Dhuism et al. 2010).

### 2.2. Ecological function of ponds

Ponds can be either created by natural processes such as glaciation, river action, large tree fall, or have a man-made origin (Oertli et al. 2005). Some are the result of mineral extraction (*e.g.* gravel quarry), while many other were created to support human activities (*e.g.* fish ponds, water storage). The main use of ponds is water supply used for agriculture, protection against fire or industrial cooling and washing. Many ponds were also created for fish production, recreational or ornamental uses (Oertli et al. 2005). Although their surface may seem insignificant at the scale of a landscape, these small waterbodies can provide a large panel of ecological services like pollution control or water flow regulation (Céréghino et al. 2014). Pond ecosystems are able to recycle nutrients that are produced by agriculture (*e.g.* nitrogen and phosphorus) and brought by runoff from the surrounding lands (O’Geen et al. 2010; Díaz et al. 2012). Along with wetlands, they also participate in the storage of floodwater and retain pollutants like metals or pesticides, protecting the integrity of downstream waterbodies (Biggs et al. 2017).

### 2.3. Ponds as biodiversity hotspots

Besides these ecological services, ponds have a considerable value for biodiversity, in terms of richness and rarity compared to all other waterbodies with respect to the same area (Søndergaard et al. 2005; Davies et al. 2008; Martinez-Sanz et al. 2012; Hill et al. 2016; Biggs et al. 2017). The high biodiversity on ponds is essentially related to the large panel of vegetation available in these ecosystems, providing rich aquatic habitat structures and food (Fig. 7; Brönmark & Hansson 2005). The bottom of the pond, is covered with sediment, organic debris or leaf litter and can host molluscs (*e.g.* planorbids, gastropods), oligochaetes, chironomid larvae and crustacea (*e.g.* Copepoda, *Gammarus* sp.) that participate in litter degradation. From the pond bottom, submerged hydrophytes (*e.g.* *Utricularia* sp., *Ranunculus* sp.) colonise the pelagic zone, where they can host aquatic insects (*e.g.* dragonflies, mayflies, beetles) living between the leaves, and amphibians that used them as a substrate for their eggs. Floating hydrophytes (*e.g.* *Potamogeton natans*, *Lemna* sp.) develop their leaves at the water surface. They enable aquatic larvae to hide from terrestrial predators. Other flying insects like damselflies may also land on their surface to mate and lay eggs. Helophytes (*e.g.* *Phragmites* sp., *Juncus* sp.) grow from their underwater roots and produce leaves above the surface. Since they are often close to the banks and form an interface between water and air, they enable many exchanges between aquatic and terrestrial life. For instance, they can be used as emergence support by aquatic larvae or provide ideal perches for flying insect species that monitor the water surface to find mates or chase concurrence. Therefore, many aquatic insects and amphibians use these small waterbodies in their early stages. Overall, one single pond is able to host a large panel of species that often have an important conservation value (*e.g.* amphibians; Rannap et al. 2010). At the landscape scale, the complementarity of several ponds presenting heterogeneous characteristics and located in various environmental contexts can lead to significant increases in the regional species richness (Martinez-Sanz et al. 2012).



**Fig. 7.** Schematic representation of the habitat structures and organisms living in a pond.

Due to their food habits, fishes have deleterious impacts on pond invertebrate communities by competition for prey resource or direct predation. Their presence or absence in ponds leads to a shift in the community structure called “predator transition” (Søndergaard et al. 2005; Van Allen et al. 2017). Fish are essentially present in large ponds (*i.e.* > 1 ha) because small waterbodies are more exposed to summer dry out and winter cold due to their limited depth (Søndergaard et al. 2005). They are often introduced by man for fishing (*e.g.* common perch; Wittwer et al. 2010) or recreational purposes (*e.g.* goldfish; Copp et al. 2005). Introduced fishes, along with other species like invasive red-eared slider or crayfish, may also impair the functioning of pond ecosystems (Polo-Cavia et al. 2011; Herrmann et al. 2018). For instance, most fish species prey on macro-invertebrates and their foraging activities can lead to an increase in water turbidity (Lemmens et al. 2013; Adámek & Maršálek 2013).

## 2.4. Pond loss and management initiatives

In Europe, ponds located in agricultural landscapes have long been maintained by farmers because they could provide a source of water for grazing cattle. However, rural consolidation and changes in agricultural practices led to the loss of many ponds during the past century (*i.e.* destruction or natural filling successions; Heath & Whitehead 1992). Along with this loss, the intensive use of inputs led in many places to the eutrophication of remaining waterbodies (Mandiki et al. 2014; Rosset et al. 2014; Katayama et al. 2015). Climate change, with the increase of drought events, is also a threat for many permanent ponds. Although the estimated number of ponds across the world may seem huge (c.a. 500 million), the combination of all these environmental and anthropic pressures led to a dramatic decrease during the past century (Holgerson & Raymond 2016). For instance, in Europe, pond loss was estimated to reach about 50% during the last decades with up to 90% in some regions (Oertli et al. 2005). This drastic decrease in the number of ponds, along with the pressures induced by human activity and the spread of invasive species in these ecosystems, lead to severe decline in lentic freshwater populations (Heath & Whitehead 1992; Strayer & Dudgeon 2010). Paradoxically, and despite their importance for biodiversity conservation, ponds are still little studied compared to other freshwater ecosystems (Le Gall et al. 2016; Biggs et al. 2017). For instance, more research is still needed to understand the functioning of ponds, their interactions with other ponds as a network, and how to maintain barriers to non-native species that have a negative impact on the ecosystem (Biggs et al. 2017).

To compensate for the pond loss in rural landscapes, restoring or creating new ones is relatively simple (*i.e.* techniques are well developed) and cost effective due to their limited size (Williams et al. 2008b). Restoration measures essentially consist in removing overhanging trees and digging accumulated sediments (Janssen et al. 2018). These works increase insolation on the ponds and prevent their terrestrialization, favouring the presence of macrophytes and invertebrate diversity (Sayer et al. 2012). Creating ponds, even in urban areas, is also a good means to support freshwater biodiversity (Hassal & Anderson 2015; Hill et al. 2017; Sun et al. 2018). Moreover, urban ponds may also play a role in raising awareness on freshwater ecosystems and conservation issues (Hassall et al. 2014). The localisation of the ponds and connectivity between them is also a crucial issue to consider in planning and management policies (Hill et al. 2018).

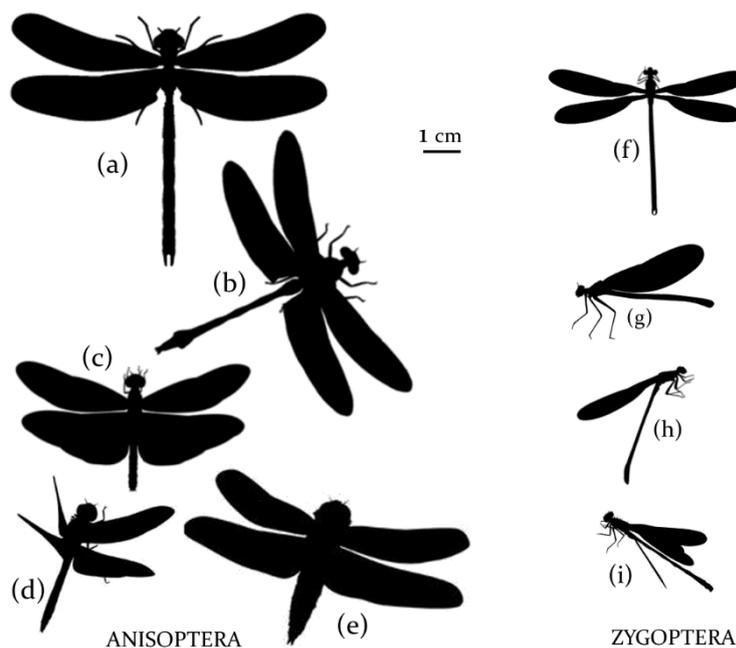
## 2.5. Maintaining connectivity between these scattered ecosystems

Since ponds are very small and scattered patches in the landscape, the connectivity between several ponds is determinant to allow gene flow and *a posteriori* population maintenance (Incagnone et al. 2015; Tigano & Friesen 2016). Pond ecosystems are therefore often interacting with each other to form a large network called “pond network” (Ribeiro et al. 2011; Thornhill 2018). Exchanges of individuals between ponds occur through different processes depending on the studied species. Many aquatic insects and amphibians use water only for their larval development and have a terrestrial adult stage (Rothermel et al. 2004; Bried & Ervin 2006). For these species, dispersal occurs during the terrestrial adult life, although some cases of transportation of eggs or larvae by birds were also reported (Bilton et al. 2001). Depending on their dispersal capacities, freshwater species will sometimes not be able to disperse to another pond, leading to the isolation of their populations (Serrano et al. 2020). The composition of the terrestrial matrix between ponds also conditions the movements and survival of individuals (Šigutová et al. 2017; Cayuela et al. 2019). The distance between ponds and the composition of the landscape matrix between them are therefore two essential components to consider in the conservation of pond networks (Pedruski et al. 2011; Guerry & Hunter 2002).

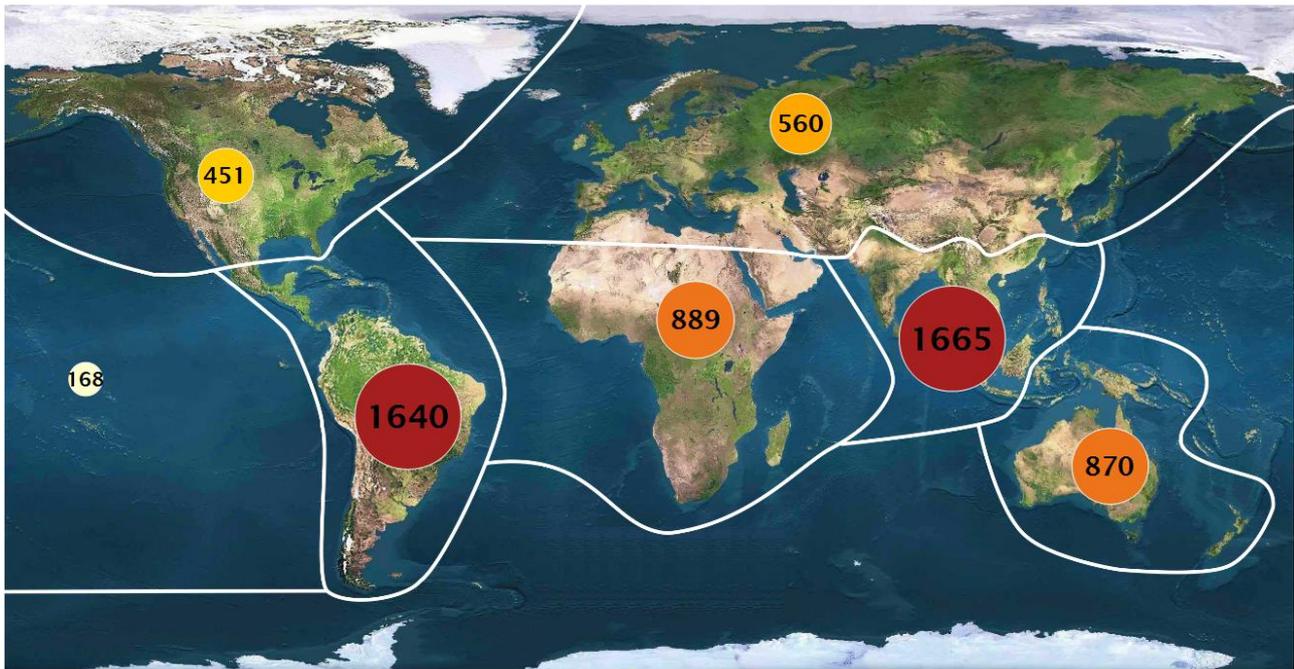
## 2. Dragonflies: A model for dispersal studies

### 3.1. Evolution of odonates and current diversity

Dragonflies belong to the taxonomic order of odonates and are often presented as insects with aquatic larvae and flying adults divided into two sub-orders: Anisoptera and Zygoptera, also called ‘true dragonflies’ and ‘damselflies’. Anisopteran species have often a robust habitus and their two pairs of wings have a different shape (*i.e.* from greek “anisos”: unequal and “pteron”: wing; Fig. 8). On the contrary, zygopteran species have a slender habitus and all their wings have the same shape (*i.e.* from Greek “zygo”: even; Fig. 8). Most of the 6,000 odonate species are concentrated in intertropical zones where a high speciation occurred during the long history of evolution for this group (Fig. 9; Sánchez-Herrera & Ware 2012). On the contrary northern temperate territories were first colonised after the last glaciation (ca. 10 000 years ago), resulting in a much lower degree of speciation (Sternberg 1998). According to the assessment of the International Union for the Conservation of Nature, about 15 % of the species may be threatened by extinction (Sánchez-Bayo & Wyckhuys 2019).

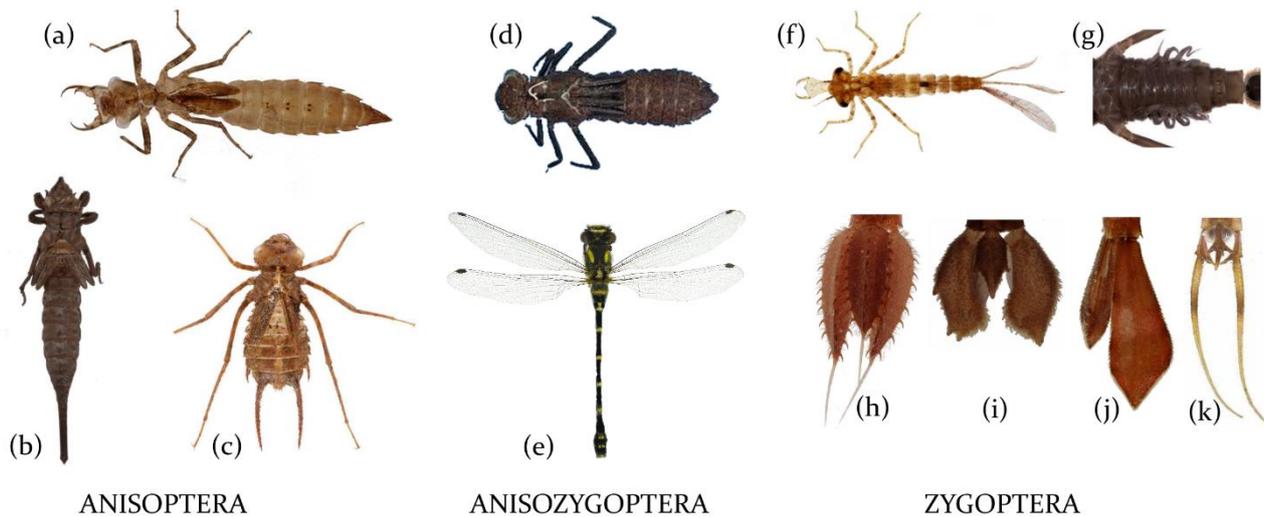


**Fig. 8.** Examples of general habitus for the main families of Anisoptera: Aeshnidae (a), Gomphidae (b), Libellulidae (c,d,e), and Zygoptera: Lesdididae (f), Calopterygidae (g), Coenagrionidae (h,i).



**Fig. 9.** Number of dragonfly species per biogeographical region (based on Kalkman et al. 2008).

The oldest fossils of Odonatoptera, date from the namurian Carboniferous (ca. 320 Myr ago; Bybee et al. 2016). This group, very close to the present odonates, is one of the oldest insect orders. It is also the first group of winged insects. Meganisoptera from the late Carboniferous were the largest species with a wingspan up to 73 cm (Kukalová-Peck 2009). If the size of the odonates is clearly less impressive today, the adult morphology has little changed. Concerning the larvae, no fossil evidence is available before the beginning of the Mesozoic (ca. 250 Myr ago). Several authors suggest that juvenile dragonflies were strictly terrestrial until the Lower Permian (ca. 275 Myr ago; Sánchez-Herrera & Ware 2012; Bybee et al. 2016). Nowadays, the majority of larvae are aquatic except for the Australian genera *Pseudocordulia* and *Antipodophlebia* in which larvae live in leaf litter (Watson & Theischinger 1980; Watson 1982). Major morphological changes among odonates occur at the level of the family and are particularly visible on the larval stages. Anisopteran larvae have all internal rectal gills supplied with water by abdominal pumping (Fig. 10a). Some of them have a long siphon that enable them to pump water when they are hidden in the mud (Fig. 10b). They often bear spines as a mechanical protection from predation (Fig. 10c). On the contrary, most zygopteran larvae have external gills, which present a wide variety of shapes and position on the abdomen (Fig. 10f,g,h,i,j & k), depending on their family. The Asian genus *Epiophlebia* has larvae and adults that resemble anisopterans, but all 4 wings are identical (Fig. 10d & e). This genus was therefore classified as a new odonate suborder called Anisozygoptera.



**Fig. 10.** Examples of larval stage of Anisoptera, Anisozygoptera and Zygoptera and an adult of Anisozygoptera. Detail: exuviae of *Neuraeshna costalis* (a), *Aphylla* sp. (b), *Aeschnosoma forcipula* (c); *Epiophlebia superstes* larva (d; [tombon.com](http://tombon.com)) and adult (e; [Hovmöller 2006](#)); *Ischnura* sp. (f), abdomen of *Chalcopteryx scintillans* in ventral view (g; [Hamada et al. 2014](#)); proctes of *Oxystigma* sp. (h), *Chalcopteryx seabrai* (i) and *Hetaerina* sp. (j), *Rimanella arcana* (k).

Overall, Odonata is a very ancient group of insects categorized today into 3 sub-orders in which most, but not all of the known species of the world have an aquatic larval stage. In the region Normandy, located at temperate latitudes, the diversity of regularly breeding odonates reaches 52 species ([biodiversite.normandie.fr](http://biodiversite.normandie.fr); [CERCION 2017](#)), whose biology and ecology is fairly well documented.

### 3.2. A life cycle between aquatic and terrestrial habitats

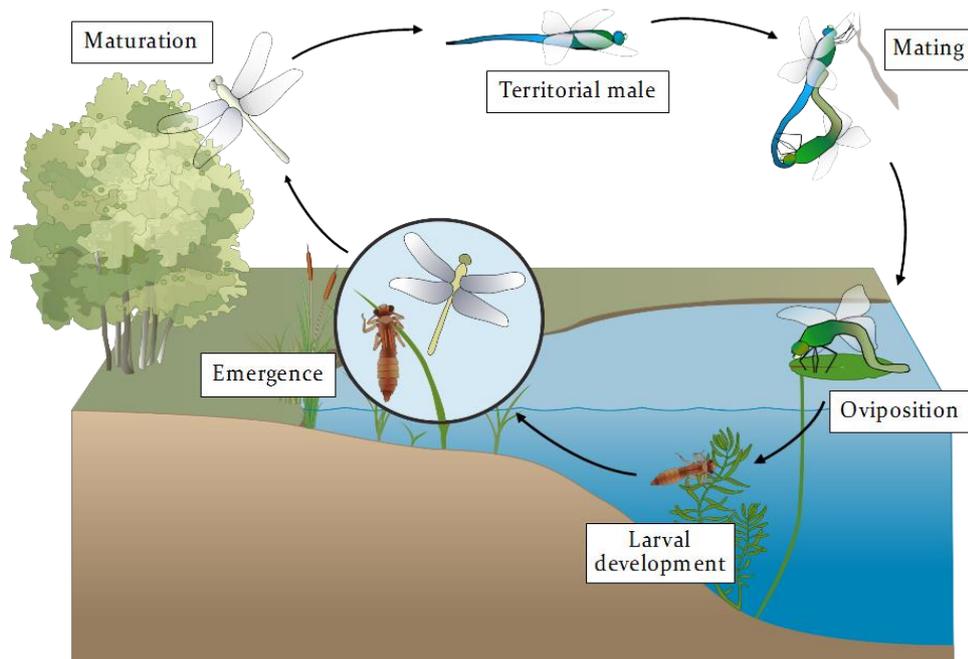
Like other aquatic invertebrates, dragonflies have a strong tropism for waterbodies, in which their larvae grow. At temperate latitudes, the larval development lasts between few months to more than 5 years, depending on the temperature and food availability ([Norling 1984](#); [Corbet 2006](#)). The number of larval instars ranges from 8 to 18 and can vary even within the same population ([Corbet 2002](#)). Eggs are laid by females either directly into the water or on a dry substrate and will hatch when the water level rises. After an eventual diapause, small prolarvae come out and rapidly undergo a first moult. They grow feeding on live preys like *Daphnias*, *Ephemeroptera*, *Chironomidae* or other smaller odonate larvae, even conspecific individuals ([Suhling et al. 2015](#)). Occasionally, large dragonfly larvae may also be able to catch small fishes and tadpoles ([Heidemann & Seidenbusch 2002](#); [Ramamonjisoa et al. 2018](#)). Preys are caught by an ejectable labium bearing two palps. The labium is either spoon-shaped and denticulated in larvae that often hunt in sediments, or flat and hooked in larvae that catch

preys visually (Heidemann & Seidenbusch 2002). Since the number of instars is variable, they are usually identified by counting from the ultimate instar labelled F0, to smaller ones: F-1, F-2, etc. (Ferrerias-Romero & Corbet 1999). During the last instar, the larva starts an internal metamorphosis under its cuticle: the eyes expand and merge, the labium shrinks and the wings grow, causing an inflation of the wing-pads (Corbet 1957). Once ready to emerge, the larva climbs out of the water on a helophyte, wood stick or rock. Some larvae may even walk up to a hundred meters far from the pond to avoid predation (Heidemann & Seidenbusch 2002). During the final moulting, the imago comes directly out of the larval skin (*i.e.* hemimetabolous cycle) and leaves behind an empty exuvia<sup>7</sup>. Dragonflies are very vulnerable during this phase, with a mortality rate between 5% and 30% (Jakob and Suhling, 1999). About 30 minutes to one hour later, the young adults, called teneral, are ready to take off. They will soon disappear in the landscape matrix, often avoiding the waterbodies for several days (LeNaour et al. 2019). During this period that may last up to two weeks, adults feed a lot on small flying invertebrates and acquire sexual maturation (Corbet 1957).

Once sexually mature, adults come back to water bodies for mating. Males are often the first arriving to the reproduction sites where they wait for females (Corbet 1999). In many species, males exhibit a territorial behaviour with a large panel of strategies to compete with other males and increase their mating success (Suhonen et al. 2008; Rivas-Torres et al. 2019). On the contrary, females spend more time feeding and roosting in the surrounding matrix and come to the water only to mate and lay eggs (Beirinckx et al. 2006). When a female arrives on a waterbody, males come and quickly grasp it with their anal appendages. If she is ready to mate, the female bends its abdomen to form a mating wheel. This copulatory position enables odonates to fly during the mating process. Copulation lasts from few seconds to several hours depending on the species (Corbet 1999). Oviposition will start shortly after mating. In most cases, the male monitors the female, preventing conspecific males to come. In several species the male still holds the female, forming a tandem during oviposition. Females are able to detect several cues (*i.e.* visual and tactile assessment of the substrate) that help them to choose favourable sites for larval development (Wildermuth 1992). Eggs may be simply dropped into the water or inserted into plants (*i.e.* endophytic), wood or soil. The general life cycle of odonates is summarized in Fig. 11.

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<sup>7</sup> exuvia: larval exoskeleton that remains after emergence of the adult



**Fig. 11.** Schematic representation of the life cycle of dragonflies with the example of *Anax imperator*.

### 3.3. Morphological traits

Dragonflies are very charismatic and attractive for the public due to their amazing colours and behaviour (Barua et al. 2012). This order was therefore quite well studied compared to other insect groups (Corbet 1999; Kalkman et al. 2008). Since both larvae and adults are quite easily found at the waterbodies and convenient to handle, they were also often used as model species in ecological studies on behaviour, functional trait-response, population dynamics or dispersal (Córdoba-Aguilar 2008; Bried & Samways 2015).

#### *Coloration patterns*

One of the most striking characteristics of dragonflies is their beautiful and various colours that play a role in sexual recognition and thermoregulation processes. In many species, immature males have the same colour as females, but their coloration will then change during the maturation process. Therefore, as in many other animal species, dragonfly colours are determinant for sexual recognition (Moore et al. 2019). Coloration is also crucial for the thermoregulation of these ectothermic organisms. Since darker colours absorb more light, they permit a greater heating of the body in environments with low temperature. That is why forest dragonfly species often have a general black coloration (e.g. tiger pattern of Cordulegasteridae), whereas species living in open areas will

exhibit a brighter coloration (*e.g.* red or blue colour and pruinosity of Libellulidae; Damm et al. 2010). Dragonflies also exhibit various behaviours to optimize their thermoregulation. Some species can perform an endothermic warm up before taking off by wing-whirring (May 1976). Many Libellulidae, can also change the position of their abdomen and wings to regulate their exposure to sun and the amount of energy they absorb (Dell' Anna et al. 1990; May 2017).

### *Sexual size dimorphism*

Aside from these differences in coloration patterns, most dragonflies also present a size dimorphism between sexes. This Sexual Size Dimorphism (SSD) can be either male or female biased, depending on the species and their mating system (Serrano-Meneses et al. 2008a). However, it seems that no general rule to understand SSD mechanisms in dragonflies was found yet (Bybe et al. 2016). Some species already show a SSD at the larval stage, but adult SSD may also come from sexually monomorphic larvae (Serrano-Meneses et al. 2007). In territorial species, male-biased SSD is often explained by the fact that larger males have more developed muscles and thus more power to fly. On the contrary, in species engaging in aerial fights, male agility favoured by a small size may be an advantage, resulting in a female-biased SSD (Serrano-Meneses 2008b). Most Zygoptera seem to exhibit a male-biased SSD (De Block & Stoks 2007; Serrano-Meneses et al. 2008b). However, in few territorial zygopteran species, males with a small body size show a higher mating rate resulting in a female-biased SSD (*e.g.* *Lestes viridis*, De Block & Stoks 2007). In Anisoptera, there doesn't seem to be a real link between male-biased SSD and territoriality (Serrano-Meneses 2008a). Observed SSD patterns may result from a differential niche utilization (Serrano-Meneses 2008b), or a fecundity selection on females that counteracts in some cases the territorial selection pressure on males (Serrano-Meneses 2017). Overall, SSD seems mostly to depend on the mating system (*i.e.* territorial or not) and the suborder, but it also seems strongly affected by other environmental factors like seasonality, and no general rule for odonates can be drawn (Wong-Muñoz et al. 2011).

### *Influence of environmental factors on morphological traits*

Some morphological traits may differ between populations according to the landscape structure. For instance, the wing size of *Calopteryx maculata* was found to be higher in open landscapes than in forests (Taylor & Merriam 1995). The body size of *C. splendens* was also shown to vary at the drainage scale of the Loire river, with larger body size at higher values of water pH and temperature (Chaput-Bardy et al. 2007). Environmental perturbations during the larval development

can affect the larval traits and *a posteriori* adult condition by a carry-over effect<sup>8</sup> (Stoks & Córdoba-Aguilar 2012). For instance, a development constraint by time (*e.g.* drought events) and low food availability will result in a lower mass at emergence (De Block & Stoks 2005). These poorly documented effects have serious implications on adult survival, fecundability or flight performance that may lead to changes in population dynamics and dispersal abilities (Stoks & Córdoba-Aguilar 2012). Food availability and water temperature are the two main factors conditioning the number of instars and the duration of larval development (Corbet 1999). Growth rate increases with temperature until it reaches an optimum. Beyond the optimal temperature (*i.e.* between 21°C and 31°C), growth rate decreases toward a lethal threshold, below which some larvae start to die (Suhling et al. 2015). Due to different climate conditions, body size of organisms changes according to latitude following Bergman's Rule that predicts larger body sizes at higher latitudes (Chown & Gaston 2010; Salewski & Watt 2017). However, in dragonfly populations body size and wing length often follows a converse-Bergman's rule with significant decrease in body size and wing length with increasing latitude (Johansson 2003, Hassall 2015) and altitude (Casanueva 2017). This can be explained by the slower larval growth at low temperatures that lead to smaller individuals but also by the fact that flight performance is reduced at lower temperatures favouring smaller species (Dudley 2002). In some species this effect might be masked by a change in voltinism. When the mean temperatures are too cold, individuals may take one more year to complete their development (Johansson 2003). Finally, the seasonality also affects the body size of dragonflies because individuals emerging later in the season probably have to shorten their larval growth to proceed with the metamorphosis (Hardersen 2010).

The presence of predators like fishes or larger dragonfly species represent a major threat for larvae. Several studies showed that even non-consumptive predators can impair the larval development. Indeed, predator presence is detected via visual clues by the prey, causing an increase in oxidative stress (Janssens & Stoks 2013). Many species living in standing water bodies have developed long spines on their abdomen (*e.g.* Fig. 10c). The length of these spines can be correlated to the size of the waterbody and the presence of fish (Johansson & Samuelsson 1994; McCauley et al. 2008b). In fact, dragonflies that live in large waterbodies are more exposed to predation and bear longer spines that species less susceptible to be preyed upon (Petrin et al. 2010). Moreover, combination between several environmental factors can act in synergism and have underestimated consequences on larval populations. For instance, the combined action of pesticides and predators leads to a greater mortality by predation because pesticides cause a reduction in escape speed and an increase in the activity level

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<sup>8</sup> carry-over effect: effect in which an environmental condition in one life stage changes fitness-related traits in the next life stage (Stoks & Córdoba-Aguilar 2012)

of the larva, making it more vulnerable (Janssens & Stoks 2013). The colours of larvae also play a role for the camouflage and allow dragonflies to hide from their predators (Henrikson et al. 1993). Within the same species, some larvae are able to change their body colour to increase their camouflage (Henrikson et al. 1993; de Oliveira & De Marco Jr 2009). Finally, larval coloration may also change across moulting during the larval development. For instance, early instars of *Anax* larvae, that are the most vulnerable to predation bear black and white bands on their abdomen, that disappear with the successive moults (Corbet 1957).

### 3.4. Ecology of dragonflies

#### *Abiotic factors shaping odonate species assemblages*

The abiotic and biotic factors shaping dragonfly species assemblages can be summarised using the filtering framework. At continental scale, the geographic distribution of dragonflies in Europe follows a general latitudinal pattern from Mediterranean to boreal species (Kalkman et al. 2018). The range size of species and their regional distribution is mainly driven by dispersal related traits like wing morphology (Outomuro & Johansson 2019). Some large species like *Anax imperator* have a distribution ranging from Southern Africa to Northern Europe, whereas other smaller species present only restricted distribution areas (e.g. relictual populations of *Coenagrion hylas* in the Northern Alps; Dijkstra 2015). In each regional pool of odonates, habitat requirements differ among species and condition the observed differences in local assemblages (McCauley et al. 2008b, Le Gall et al. 2018). Since the larval development lasts much longer than the adult life, most constraints to distribution and abundance of odonates involve the larval stage (McPeck 2008). Moreover, the autochthony of a species cannot be inferred from the presence of adults only, because they may sometimes disperse to habitats where they do not breed (e.g. *Calopteryx spp* feeding on ponds; Raebel et al. 2010). On the contrary, the presence of larvae, and even more the presence of exuviae, can assess that all ecological requirements are met to enable the full development of a species (Raebel et al. 2010). As other aquatic species, odonates face a trade-off between oxygen demand and water temperature during the larval stage. They can either develop in streams with a high level of dissolved oxygen, or benefit the generally warmer water temperature of ponds and lakes (Lopez et al. 2019). In most species (58 % of the European species), the larvae can develop in lentic water bodies<sup>9</sup> only, whereas fewer species (28 %) inhabit lotic water bodies<sup>10</sup> only (Hof et al. 2006). Some odonates (14 % of the European species) are also able to breed in both lentic and lotic waterbodies (Hof et al. 2006). The size of the water body and its propensity to dry out during summer is also a main driver of species selection because it constrains the duration of the larval stage (McCauley et al. 2008a). This constraint may benefit some species like *Lestes dryas* which develop faster than other species and take advantage of the low concurrence in temporary ponds (Stoks & McPeck 2003). The site selection is made by adults according to the context of the waterbody, especially the amount of tree cover in the surrounding and associated shading (French & McCauley 2018). The degree of sun exposure also shapes the vegetation structure and abundance which in turn conditions the dragonfly species composition and abundance (Janssen et al. 2018; Huikkonen 2019). All these factors constitute an abiotic filter selecting the ecological pool of

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<sup>9</sup> lentic water body: body of standing water, ranging from ditches, seeps, ponds, seasonal pools, basin marshes and lakes (Reinbold 2018)

<sup>10</sup> lotic water body: any kind of moving water, such as a run, creek, brook, river, spring, channel or stream (Reinbold 2018)

species that can inhabit a waterbody. The vegetation structure can here be considered as an abiotic habitat factor, little biotic interaction occurs between plants and dragonflies.

### *Biotic factors shaping odonate species assemblages*

Biotic interactions represent the last level of constraints shaping odonate species assemblages and abundance. Predation, either by fish, larger odonate species or cannibalism can be responsible for up to 80% of the larval mortality (McPeck 2008). The presence of fish may reduce larval abundance and lead to the exclusion of some species which large and active larvae are easy to prey (Johansson et al. 2006; McPeck 2008). At many sites, odonate populations remain constant over years suggesting that a density dependent population regulation is occurring even in absence of predators (Crowley et al. 1987; Van Buskirk 1987, 1993). This regulation is likely to be associated with competition for food that limits population growth, and encourages cannibalism when the amount of prey is limited (Van Buskirk 1989; Johansson & Crowley 2008). Some parasites like water mites also affect the development of larvae but their impact on abundance remains poorly documented (Zawal & Buczyński 2013). At the adult stage the biotic factors shaping species associations and their abundance are less known (McPeck 2008). Population abundance relies mostly on the fecundity of females, which depends on the number of times they will oviposit in the waterbodies (Bennett & Mill 1995). Their ability to avoid predation and to forage during the maturation of eggs must therefore be a determinant criterion for driving population demography. Some parasites like water mites were also shown to reduce fecundity but do not seem to affect the survival of adults (Rolff 1999; Córdoba-Aguilar 2006). There might also be a density-dependent population regulation in adults via competition and an increase in parasitism with population size, but these mechanisms remain poorly known (McPeck 2008).

### *Metapopulations dynamics*

The approach using the filtering framework provides a robust basis to describe species assemblages and abundance, but does not account for the dynamics between populations. Several studies showed that species assemblages of odonates on ponds present a strong autocorrelation with pond surroundings up to 13 km (Briers & Biggs 2005; Hamasaki et al. 2009; Thornill et al. 2018). Thus, the similarities between close populations are at least partly driven by metapopulation dynamics and source-sink interactions (McCauley et al 2008). Especially, the importance of dispersal and connectivity between populations were found to increase with the intensity of perturbation events (e.g. summer droughts; McCauley et al 2008). Metapopulation dynamics enable to maintain the structure of species assemblages over time through re-colonisation after extinction events. For

instance, a study on *Sympetrum obstrusum* highlighted a strong effect of the year of sampling on the population genetic structure indicating that a high turnover rate is occurring on ponds across the years (Phillips & Swanson 2018).

### *Terrestrial habitats during adult life*

Odonates differ from other aquatic insects by their ability to exploit also terrestrial habitats far from their natal sites (Foster & Soluk 2006). They spend a lot of time away from the ponds to rest and prey on a wide variety of small flying insects and some non-flying arthropods (*e.g.* aphids; Baird & May 1997). Their movements are therefore driven by the amount of food available in the different terrestrial habitats. The foraging behaviour of dragonflies sometimes leads them to fly several kilometres away from the nearest waterbody (Popova et al. 2017; Hykel et al. 2019). Their need of a large amount of preys in the vicinity of the waterbodies where they breed make odonates good indicators of the quality of the terrestrial surroundings (Chovanec & Waringer 2001; Oertli 2008; Berquier et al. 2016).

Due to their mating behavior, the males must spend more time on ponds than females to defend a territory (Suhling 2015). Hence, females have more time to forage in the surroundings of waterbodies in order to accumulate the energy required for the development of their egg clutches (Beirinckx 2006; Foster & Soluk 2006). However, while the activity patterns of odonates, and especially males, were well studied on the breeding sites, little is known about their behaviour and habitat use in terrestrial habitats (Hykel et al. 2016, 2018; Le Naour et al. 2019). The home range area was characterized only in four anisopteran species (Che Salmah 2000; Levett & Walls 2011; Dolný et al. 2014; Moskowitz & May 2017) and one zygopteran species only (Ward & Mill 2007). Moreover, these studies often remain incomplete because of their short study-time duration or their focus on one sex only (Levett & Walls 2011; Moskowitz & May 2017). Results showed that terrestrial requirements could be up to 1000 times greater than the area of the waterbody in which the larval development occurred (Dolný et al. 2014).

Some landscape features were shown to drive dragonfly survival and movements during their terrestrial life. For instance, the presence of motorways was identified as a barrier that prevents dragonfly movements between habitat patches probably by increasing mortality of crossing individuals (Šigutová et al. 2017). Road mortality was shown to be very variable between dragonfly species and affected especially more the low flying species (*i.e.* under 2 m) such as *Plathemis lydia* or *Libellula luctuosa* than others (Soluk et al. 2011). Visual cues like linear rows of hedges may be used as leading structures to guide movements in the landscapes while forest patches act as potential barriers (Faubet & Gaggiotti 2008; French & McCauley 2019). However, these hypotheses were rarely tested,

and a study on the dragonfly *Leucorrhinia caudalis* found that movements could not be explained with landscape elements on a study scale of 10 km (Bolliger et al. 2011). Therefore, the role of the landscape composition on dragonfly terrestrial activities, survival and movements still requires more investigation.

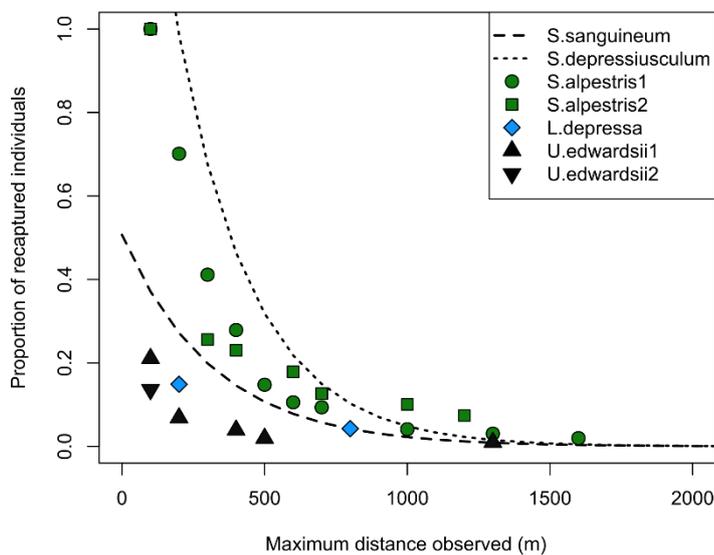
### 3.5. Dragonfly dispersal

#### *Dispersal distances*

About 100 species of Anisoptera may be able to undertake migrations on long distances (Russell et al. 1998). The most common example is *Pantala flavescens* which has the longest known migration of any insect (Troast et al. 2016). It can cover up to 4,000 km across the Indian Ocean helped by the wind currents (May 2013). In Europe, some species like *Leucorrhinia pectoralis* undertake occasionally massive migrations from Eastern populations to Western Europe (Goffart et al. 2012; Lorthois 2013). Such migration events are difficult to study because they are rare and seem unpredictable (Russell et al. 1998). Besides these intended migrations, some individuals are also sometimes detected very far from their normal distribution range. It is the case of the African Aeshnid *Hemianax ephippiger*, that was observed several times on the South American coast (Lambret & Boudot 2013). Such rare events of long-distance dispersal are also reported for Zygoptera. For instance, *Ischnura hastata* was found in the Azores more than 3,000 km far from the nearest population. The origin of the population is unknown, but it was probably brought by the wind as this species is able to fly at high altitudes (*i.e.* 300 m; Cordero-Rivera et al. 2005). Nevertheless, these “accidental” long distance movements remain very scarce and most dragonfly species seem unable to undertake migrations (Corbet et al. 1999; Suhling et al. 2017).

After their emergence from the larval envelope, adult dragonflies take their first flight (*i.e.* maiden flight) and often avoid waterbodies during several days until they become sexually mature (Corbet 1957; Le Naour 2019). Little is known about their behaviour during this period and the natal dispersal of dragonflies. In Zygoptera, some species are probably dispersed by the wind during their maiden flight (Thompson 1991). In Anisoptera the rate of philopatry may be very variable among species even within the same genus (*e.g.* *Sympetrum spp.*; Dolný et al. 2013). Only few data are available about the natal dispersal distances since most individuals probably fly out of the detection range during the first days (Hardersen 2007; Le Naour 2019). No difference was reported between males and females during this period (Hardersen 2007). After maturation, individuals come back to the ponds to breed. Most individuals stay on the same pond during their entire lifetime, whereas others will

reproduce in several different ponds, leading to breeding dispersal (Conrad et al. 1999; Rouquette & Thompson 2007; Dolný et al. 2013; Le Gall et al. 2017). Many studies reported dispersal events between different waterbodies separated by more than one kilometre for Anisopteran species (e.g. Chin & Taylor 2009; Kéry & Juillerat 2004; Knaus & Wildermuth 2002). However, these distances increase with the sampling effort and are often limited to the extent of the study area (Schneider 2003; Hassall & Thompson 2012). Although not exhaustive, these data can be used to model the distribution of the movements observed and infer the probability of moving a certain distance to compare dispersal abilities between species (Fig 11 & Table 1)



**Fig. 11.** Probability of movement of Anisoptera. Only movements of lentic species between waterbodies are presented.

**Table 1.** Details and source of the studies used in Fig. 11.

Species name	Sampling year	Reference
<i>Sympetrum sanguineum</i>	1997	Conrad 1999
<i>Sympetrum depressiusculum</i>	2012	Dolný et al. 2014
<i>Somatochlora alpestris</i> (1)	1998	Knaus & Wildermuth 2002
<i>Somatochlora alpestris</i> (2)	2000	Knaus & Wildermuth 2002
<i>Libellula depressa</i>	2001	Angelibert & Giani 2003
<i>Urothemis edwardsii</i> (1)	2016 (Lac Bleu)	Khelifa et al. 2016
<i>Urothemis edwardsii</i> (2)	2016 (Lac Noir)	Khelifa et al. 2016

### Factors shaping dispersal patterns

Anisoptera are generally larger and more robust species, they have therefore better dispersal abilities than the smaller Zygoptera (Conrad et al. 1999; Suhling et al. 2017). Although dispersal abilities differ among species, the movements of dragonflies are also shaped by intraspecific variations in traits. Within the same species, morphological traits like wing shape (Conrad et al. 2002) or body size (Anholt 1990; Michiels & Dhont 1989) were sometimes found to be correlated with dragonfly dispersal. However, these relationships do not seem to work as a general rule in all species (Chaput-Bardy et al. 2010). Differences in behaviour may also impact whether individuals will disperse or not. Just after emergence, they spend several days away from the ponds before they acquire sexual maturation. Little

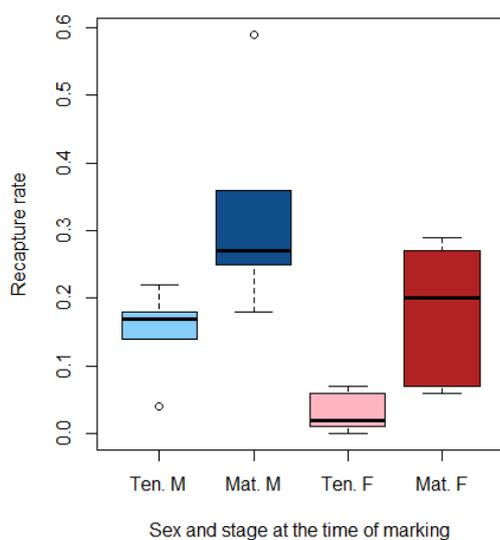
is known about this part of the life of dragonflies, but several studies suggest that there might be a higher dispersal rate during this period (Crumrine et al. 2008). For instance, mean natal dispersal was reported to be higher (*i.e.*  $596 \pm 5$  m) than breeding dispersal (*i.e.*  $181 \pm 239$  m) in *Gomphus lucasii* males (Zebsa et al. 2015). During the mature life, some males called “residents” (Lancaster & Downes 2017) will establish on a breeding site to defend a territory, while others called “itinerants” (Lancaster & Downes 2017) will not be able to establish and move frequently between sites (Higashi 1969, Koenig & Albano 1987; McCauley 2010). This pattern is less clear for females, but in some cases, they may decide to leave their breeding pond to avoid male harassment and lay eggs in a quieter waterbody (McMillan 2000). The overall difference between male and female dispersal remains unclear and may vary among species. Due to their activity patterns, females spend more time away from the ponds and are more prone to dispersal (Beirinckx et al. 2006). However, in some cases, males that have lower energetic flight costs due to their lower body mass might be more inclined to undertake long distance dispersal movements between waterbodies (Damm & Hadrys 2012).

The dispersal is often density dependant because high densities increase competition for resource, territory and sexual harassment (Clobert et al. 2012). In some dragonfly species, migration movements can be induced by excessive population growth (Kharitonov & Popova 2011). However, many species also tend to aggregate at the most favourable sites which often leads to a negative relationship between population density and dispersal movements (Rouquette & Thompson 2007; Chaput-Bardy et al. 2010; Allen & Thompson 2010). The presence of parasites may also increase dispersal because it increases the energetic requirements of individuals and leads them to spend more time foraging away from the ponds (Conrad et al. 2002). The dispersal behaviour is linked to the age of dragonflies in some species like *Sympetrum danae*, which undertakes more escape flights at an age of 25-30 days (Michiels & Dhont 1991).

Dispersal events and distances are also dependant on some abiotic factors linked to environmental perturbations or the landscape context. For instance, pond drought is often responsible for a departure of dragonfly species to other neighbouring ponds (Le Gall et al. 2017). Several studies reported an effect of composition of the landscape matrix on the mobility of dragonflies. For instance, *Ischnura elegans* and *Coenagrion mercuriale* were found to be more mobile in open agricultural landscapes than in urban areas or other landscape contexts (Le Gall et al. 2017; Keller et al. 2012). The presence of forest patches and flowing waterbodies also acted as barriers to the dispersal of *C. mercuriale* (Keller et al. 2012). The length of local movements of two *Calopteryx* species were found to be shorter in forests than in open landscapes and the probability of moving away from the stream depended on the landscape type (Jonsen & Taylor 2000a; Jonsen & Taylor 2000b).

*Methods to study dragonfly dispersal*

Wing marking is the oldest Capture-Mark-Recapture (CMR) method and the most widespread method to study dragonfly movements (Borrer 1934; Cordero-Rivera et Stoks 2008). Individuals are caught, identified with a unique code on the wing using a permanent marker or nail polish and subsequently recaptured or resighted (Cordero-Rivera et Stoks 2008). The percentage of recapture in such studies ranges from 1% to 59 % depending on the intensity of the study (*i.e.* number of individuals marked and number of recapture sessions; Fig. 12 & Table 2). In most species, the recapture rate is higher for males than for females due to their different reproductive behaviours: males are often territorial while females are more elusive to avoid harassment (Corbet 1999). This method can bring relevant information on the rate of movements between breeding sites (*e.g.* Conrad et al. 1999). However, CMR studies are very time consuming because many individuals are never resighted again and only marking a large number of individuals allows to detect these movements.



**Fig. 12.** Recapture rates of Anisoptera in CMR studies. Only studies using both marking at emergence and adult CMR were used for this graph ( $n = 5$ ).

**Table 2.** Details and source of the CMR data used in Fig. 12. The columns *n teneral* and *n adults* indicate the number of individuals used in the study.

Species name	n teneral	n adults	Sampling year	Reference
<i>Leucorrhinia pectoralis</i>	130	399	2016	Minot 2016
<i>Somatochlora alpestris</i>	92	185	2000	Knaus & Wildermuth 2002
<i>S. alpestris</i>	126	187	1998	
<i>Aeshna tuberculifera</i>	34	52	1977-1981	Halverson 1984
<i>A. umbrosa</i>	35	48		

Telemetry methods provide accurate data on individual movements even when they are hidden in the terrestrial habitats. For instance, the development of radio-tracking and especially the lightening of embedded batteries used on the radio-transmitters allow to equip and track the largest dragonfly species (Levett & Walls 2011; Moskowitz & May 2017). However, even the smallest transmitters remain too heavy (*c.a.* 0.2 g) for most dragonfly species. Therefore, small passive tags associated with harmonic radars were also developed to track smaller Anisoptera. These light tags (<

0.02 g) can be detected up to 100 meters and enable to study the natal dispersal of dragonflies (Hardersen 2007; Le Naour et al. 2019). However, contrary to the radio-transmitters, these tags do not enable identification of the individuals. Radio frequency identification (RFID) passive systems can also be used for dragonflies, but their use is restricted to species inhabiting small streams (e.g. Cordulegasteridae) due to the limited detection range of the tags (Kissling et al. 2014).

Dragonfly handling and marking during CMR studies might have an impact on their survival and behaviour. Several studies reported a lower apparent survival<sup>11</sup> on the day just after wing-marking (Cordero-Rivera & Stoks 2008). In some cases, it could be attributed to damages due to handling, especially on small species. Dragonflies are also expected to undergo a stress that leads them to avoid the waterbody where they were caught during subsequent days. This behaviour might be responsible for the lower apparent survival. Telemetry methods might also have a strong impact on individual survival and behaviour due to the transmitter mass and cluttering. However, this effect has never been tested.

On a larger scale, effective dispersal events between odonate populations can also be assessed using genetic methods. The measurement of gene flow between populations often confirms empirical field studies on dragonfly dispersal. For instance, in the rare *Coenagrion mercuriale* differences in genetic structure can be detected among sites distant from ca. 20 km (Lorenzo-Carballa et al. 2015) confirming the low dispersal capacity of this species (i.e. 50-300 m; Purse et al. 2003). On the other hand, the genetic structure of the “globe skimmer” *Pantala flavescens* could only be detected on a global scale confirming the field observations of long-distance migrations for this species (Troast et al. 2016). In some cases, population genetic studies can also bring new insights into population dynamics. For instance, individual movements of *Ischnura elegans* are seldom observed on field studies (Le Gall et al. 2017), while a high gene flow between populations was highlighted at the European scale (Wellenreuther et al. 2011). Population genetic studies also allow to detect whether isolation is due to the distance or to geographical barriers and identify populations that should be prioritized for conservation purposes (e.g. *Leucorrhinia dubia*; Johansson et al. 2017).

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<sup>11</sup> apparent survival: product of the probabilities of true survival and of study area fidelity (Schaub et Royle 2014)



# Objectives and main hypotheses

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Previous studies on dragonflies provided a large amount of data on observed movements, behaviours and mechanisms that condition dragonfly dispersal. Local movements of odonates were mostly studied using CMR on many species, providing relevant information about population exchanges between nearby waterbodies. The role of dispersal in shaping and maintaining local communities, especially in habitat exposed to a high degree of perturbation, was also elucidated. Finally, the biotic interactions conditioning survival and mating success were already explored. Nevertheless, several aspects of the life traits (*e.g.* carry-over effects from larvae to adults) and environmental factors (*e.g.* composition of the landscape matrix) that shape both local and long-distance movements of odonates remain poorly known.

The first objective of this work was to try to fill the gaps between the larval development and the effective dispersal of adult odonates. Especially, we wanted to understand to which extent the larval development could affect the condition of the adults and their ability to disperse. We were also interested in the relationship between local movements and the composition of the landscape matrix. Finally, we aimed to quantify the actual dispersal of odonates, their ability to colonise new habitats, and the genetic exchanges between populations. To address these questions, we conducted several studies from the larval stage to the movements of mature flying adults and worked from the local scale of a single pond to populations at the European scale.

**Part I: Colonisation of new ponds by dragonflies.**

*Study species: all odonates*

Odonates are mobile flying insects, whose dispersal capacity may vary a lot between the species. The sub-order Anisoptera is composed of larger and more robust species than Zygoptera and have probably better dispersal abilities. Studying colonisation of new habitats is a good way to assess the effective dispersal events of dragonflies because it implies that the species was able both to move to a new habitat and to breed. This part is composed of one chapter:

**Chapter 1:** *Pond creation and restoration: patterns of odonate colonisation and community dynamics.*

In this first chapter, we studied the colonisation of 20 new ponds by both sub-orders of odonates over 3 years. The composition of the species communities was investigated in relation to the environmental characteristics and connectivity with other ponds.

**Hypothesis I: The intrinsic dispersal capacities of dragonflies as well as environmental factors condition their ability to colonise new waterbodies.**

**H. I-a:** There is a difference in the colonisation rate of new ponds between Anisoptera and Zygoptera.

**H. I-b:** The total number of species on new ponds will increase during the first years after pond creation.

**H. I-c:** The landscape context and the local characteristics of new ponds condition species assemblages and abundances of dragonflies.

**H. I-d:** The structural connectivity between ponds also conditions species assemblages and abundances of dragonflies.

**Part II: Larval development, consequences on adult condition and natal dispersal.**

**Study species:** *Anax imperator* & *Aeshna cyanea*

Dragonflies have a long aquatic larval stage during which they can be exposed to anthropogenic or environmental factors of stress. Perturbations like water pollution may affect their development with potential consequences on the adult life. In this part, we studied the relationship between the larval traits and the condition of the adult, their survival and their propensity to disperse. We also investigated the potential stress caused by water pollutants on individuals and their consequences on larval development and adult condition. This part is composed of two chapters:

**Chapter 2:** *Biometry of the large dragonfly Anax imperator (Odonata, Aeshnidae): A study of traits from larval development to adults.*

In this second chapter, we studied the relationships between morphological traits of the last larval instars and the condition of the adults.

**Chapter 3:** *Effects of water pollution on the larval development and condition of the adults at emergence in Aeshna cyanea (Odonata, Aeshnidae).*

In this third chapter, we investigated how the physico-chemical properties of the water and especially, the presence of an herbicide (Roundup) and an insect repellent (DEET), could affect the development of the larvae and their consequences on the condition of the adults.

**Hypothesis II: Anthropogenic and environmental perturbations during the larval development can affect adult condition, survival and dispersal.**

**H. II-a:** There is a relationship between larval and adult traits in *A. imperator* and *A. cyanea*.

**H. II-b:** The traits of *A. imperator* adults at emergence condition their survival and their natal dispersal.

**H. II-c:** The exposure to pollutants in water causes a physiological stress to the larvae of *A. cyanea*.

**H. II-d:** The stress caused by exposure to pollutants has a negative impact on the physical traits of the larvae of *A. cyanea*.

**H. II-e:** The stress during the larval development affects negatively the condition of teneral adults after emergence in *A. cyanea*.

**Part III: Dispersal of the large dragonfly *Anax imperator*.**

***Study species: Anax imperator***

In this part, we studied the intrinsic and environmental factors that shape the movements of mature dragonflies. The potential barriers to dispersal and consequences on gene flow at the European scale was also investigated. This part is composed of two chapters:

**Chapter 4:** *Habitat use and movements of a large dragonfly (Odonata: Anax imperator) in a pond network.*

Since the sexual behaviour of males and females is very different in odonates, there may be consequences on their use of habitats surrounding the waterbodies. Mature females need more food to support the development of their eggs. They can also spend more time in the landscape matrix, since they don't need to stay on ponds contrary to the territorial males. In this fourth chapter, we therefore investigated if there were differences between both sexes in home range size and habitat use. We also studied movements between nearby ponds.

**Chapter 5:** *Diversity and genetic structure of Anax imperator populations at the European scale.*

Effective dispersal can be indirectly measured by the study of gene flow between populations. Since our work focused on the large and highly dispersive species *Anax imperator*, we did not expect any genetic structure within Normandy. In this last chapter, we therefore investigated the genetic structure of populations at both local and European scale.

**Hypothesis III: The characteristics of local movements and large-scale dispersal of dragonflies depend on both species' intrinsic and environmental factors.**

**H. III-a:** Mature females are more mobile and have a larger home range than males.

**H. III-b:** Mature females move more frequently between ponds than males.

**H. III-c:** Males and females select specific habitats in the landscape matrix during their mature life according to their needs (*e.g.* reproduction, hiding place).

**H. III-d:** The dense regional pond network allows a high dispersal rate and gene flow between populations in Normandy.

**H. III-e:** Geographical barriers like mountains or seas lead to genetic isolation of populations at European scale.

## Part I

# Colonisation of new ponds by dragonflies

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## **Chapter 1**

### **Pond creation and restoration: patterns of odonate colonisation and community dynamics**

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#### **Abstract**

Ponds are lentic waterbodies with a high conservation value for biodiversity that have long been disregarded by management policies. Recent initiatives aimed to promote the conservation of these ecosystems by restoring or creating new ponds thorough Europe. Studying responses of aquatic invertebrates to local pond characteristics and connectivity between them is determinant to understand community dynamics and colonisation processes of these scattered ecosystems.

We studied larval communities of odonates in 20 newly created or restored ponds to assess their colonisation during the 2 or 3 first years. Community dynamics in relation to pond vegetation, pond landscape context and connectivity with other ponds was also investigated. A total of 24 odonate species, representing about 45% of the regional species pool were identified. Most species colonised the ponds during the first year, but there was a different pattern in the colonisation rate between Anisoptera and Zygoptera. Community composition was related to local characteristics and the time since pond creation. Odonate abundances were also positively related to pond connectivity in both sub-orders and this relationship decreased with the age of ponds for Anisoptera. Surprisingly, compared to previous studies, vegetation structure had a weak impact on larval density and diversity.

This work confirmed the high colonisation capacity of odonate species and showed that creation of new ponds could be as efficient as pond restoration to enhance odonate diversity. It also highlighted the importance of connectivity between ponds, especially to support higher abundances and a posteriori increase population viability at the landscape scale.

**Key words:** Connectivity, Landscape context, Pond network, Temporal dynamics, Vegetation structure

## 1. Introduction

During the past century, the number of European ponds decreased by about 50 % and up to 90 % in some regions (Oertli et al. 2005). This loss was especially a consequence of filling, drainage and abandon of many ponds due to changes in land use driven by agricultural practices and urbanisation (Jeffries 2012). However, these small waterbodies are increasingly recognised for the important ecological services they provide (*e.g.* water retention, pollution removal) and their high value for promoting and preserving biodiversity (Céréghino et al. 2014; Biggs et al. 2017). The great variability in environmental conditions and habitat niches between ponds allows a high biodiversity outcome even in anthropized landscapes such as urban areas (Florencio et al. 2014; Hill et al. 2017). Although they are the most abundant aquatic ecosystems (Holgerson & Raymond 2016) and despite their ecological relevance, ponds have long been disregarded in land planning due to a lack of recognition (Biggs et al. 2017). Under the impulsion of the European Conservation Pond Network (Oertli et al. 2005), many initiatives aimed to promote the conservation of these ecosystems during the last decade, by restoring or creating new ponds thorough Europe (Williams et al. 2008). Moreover, since ponds are small and scattered patches in the landscape, a good connectivity between them is crucial to allow dispersal events and maintenance of populations (Kindlmann & Burel 2008; Incagnone et al. 2015). For these reasons, landscape management now regularly includes greenways and blueways to promote favourable corridors between these freshwater habitats and help connecting the populations. However, few feedbacks are available on the efficiency of these management plans (Amsallem et al. 2018).

Spontaneous pond succession dynamics occurring after creation or restoration lead new ponds to fill by sediments, making them shallower until complete drying out (Biggs et al. 1994). Management practices mostly consist in the removal of overhanging trees to increase insolation and digging accumulated sediments to avoid their terrestrialization (Janssens et al. 2018). These practices were shown to increase significantly macrophyte and invertebrate diversity (Sayer et al. 2012). The management of fish and invasive species (*e.g.* crayfish or red-eared slider) is also crucial because these organisms can have deleterious effects on pond biodiversity by both predation on the native fauna and damages on native vegetation (Polo-cavia et al. 2011; Lemmens et al. 2013; Twardochleb et al. 2013). In meadow ponds, cattle grazing and trampling destroys banks and aquatic vegetation, and may increase nutrient load and turbidity (Bagella et al. 2010; Harrison et al. 2017). These changes in trophic structure and water abiotic characteristics have negative impacts on pond biodiversity, but

can be mitigated by some management practices like adding fences to a part of the pond or practicing rotation grazing (Giuliano 2006; Silver & Vamosi 2012).

Aside from the aquatic habitats, the terrestrial surroundings of the ponds are also used by the adult stage of many amphibian and invertebrate species (*e.g.* Hill et al. 2016; Zamberletti et al. 2018). The composition and spatial organisation of the surrounding landscape of the ponds is therefore determinant for the maintenance of these species by allowing them to disperse and colonise new sites. However, colonisation of ponds is also strongly related to the dispersal abilities of aquatic organisms (Bilton et al. 2001). It depends on both the functional traits of species and the connectivity between waterbodies in the landscape (Heino et al. 2015; Incagnone et al. 2015). Structural connectivity between ponds describes physical characteristics that are easily measurable, such as the Euclidian distance between them (Chen et al. 2017), whereas functional connectivity quantifies the actual flux of organisms and is often harder to quantify (Ribeiro et al. 2011).

Aquatic invertebrates respond very quickly to the creation of new ponds, exceptional drought events or inundation (Ruhí et al. 2009; Jeffries 2011). They are therefore good models to investigate the response of biodiversity to changes in local habitat characteristics and spatial connectivity (Verberk et al. 2010; Florencio et al. 2014). Like many other aquatic insects, odonates have an aquatic larval stage and a flying adult life (Suhling et al. 2015). They can present high species richness and densities on ponds (Oertli 2002; Raebel et al. 2012a). Since they are top high predators in trophic food chains, odonates can be used as an “umbrella group” that reflects the general condition of aquatic communities (Sahlén & Ekestubbe 2001; Oertli 2008). Local species assemblage and abundance of odonates are also regularly used as biotic indices to assess habitat quality of waterbodies and their surroundings (*e.g.* Simaika & Samways 2009; Golfieri et al. 2016; Vorster et al. 2020). Odonates are divided into 2 suborders (*i.e.* Anisoptera and Zygoptera) based on the shape of their wings at the adult stage. Anisoptera are generally larger species and the adults have better dispersal abilities than Zygoptera (Suhling et al. 2017). The movements of these very mobile insects between ponds are difficult to assess because many odonate species present an elusive behavior when they are away from the ponds (*e.g.* Hykel et al. 2018; Moskowitz & May 2017). However, few long-distance movements can have significant impacts on population maintenance because they enable colonisation of new breeding sites and gene flow (Suhling et al. 2017; Jordano 2017), especially after perturbation events.

The ability of a species to colonise a new pond also depends on its habitat requirements, also called “ecological niche”. Generalist species have large environmental tolerances and can develop in

very diverse habitats (Büchi & Vuilleumier 2014). They are more resistant to perturbations and often show minor changes in community composition according to environmental variations (Batzer et al. 2004). On the contrary, specialist species have narrow environmental tolerances which often limit them to particular habitats (Büchi & Vuilleumier 2017; Mykrä & Heino 2017). The landscape context is determinant for odonate assemblages and some species can respond strongly to habitat and land use changes (Oertli et al. 2008; Raebel et al. 2012b). There is a general gradient in community composition from species inhabiting field ponds to those inhabiting forests pond, with urban ponds often hosting a mix of both (Gall et al. 2018). At the larval stage, odonate species richness depends on environmental characteristics such as pond area (Oertli 2002; Kadoya et al. 2004) or pH (Honkanen et al. 2011). Larval abundance responds to biotic interactions like competition for food resource or predation by fish (Šigutová et al. 2015; Allen et al. 2017). Diversity and abundance of odonate larvae also increases with habitat heterogeneity because community composition is mainly driven by the structure of aquatic vegetation (Janssen et al. 2018; Huikkonen et al. 2019). The community structure is therefore shaped by both local environmental variables and stochastic processes such as dispersal abilities (Arrowsmith et al. 2018).

The relationship between abundance and occupancy (*i.e.* proportion of sites in which a species was found) is widely used in ecological studies as it allows, among others, to identify species threatened with extinction and understand changes in community structure over time (Gaston et al. 2000; Martinez et al. 2017). Abundance-occupancy relationship reflects the differences in habitat niches used by species and the dynamics of metapopulations (Borregaard & Rahbek 2010). While habitat preferences are a structural component unlikely to change with time, population dynamics can lead to changes in this relationship over years (Borregaard & Rahbek 2010). Deciphering how the odonate assemblages are shaped in different contexts and how they change over time is a key to assess the efficiency of management practices on ponds and their global biodiversity outcome (Lemmens et al. 2013; Janssen 2018).

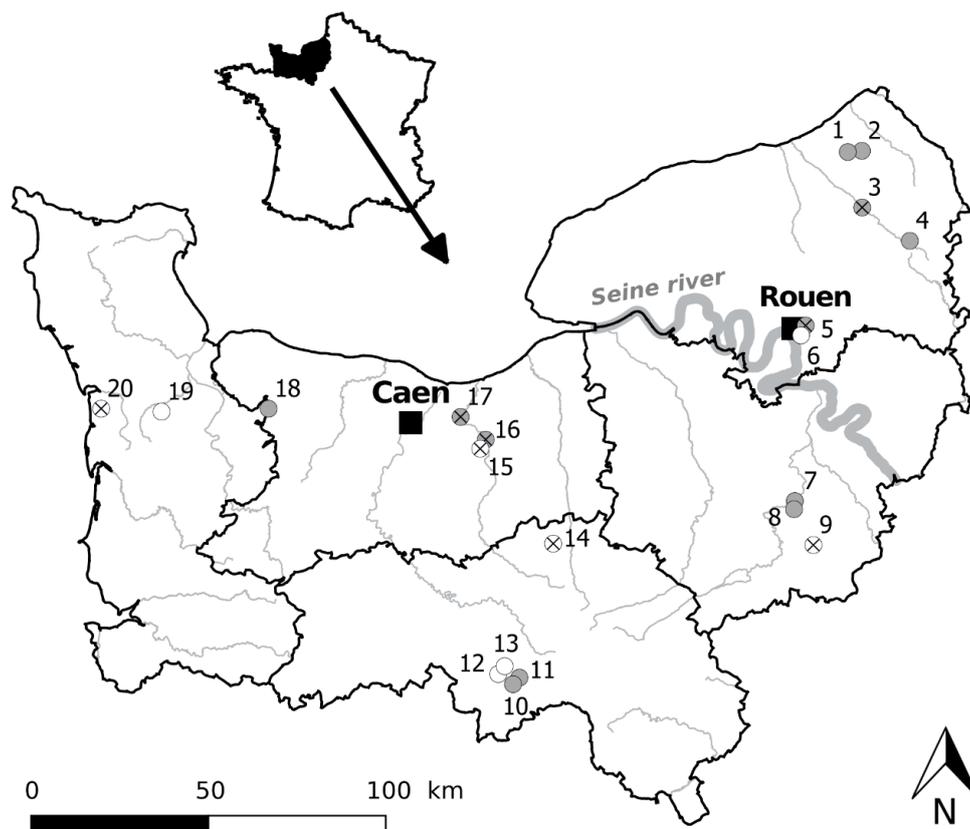
This study aimed to assess the colonisation dynamics of newly created and restored ponds by odonates and investigate their community dynamics in relation to different environmental factors. To assess the successful establishment of colonising species at the study ponds, this work focused on larval communities which were sampled each spring during 2 or 3 years on 20 ponds in Normandy (France). First, yearly estimates of species richness, number of colonising species and number of extinct species were calculated for each pond. Effects of landscape context, structural connectivity and pond age on these estimates were then tested. Especially, temporal patterns of colonisation were compared to test whether Anisoptera were better colonisers than Zygoptera. Then, we also tested

whether the community dynamics differed between created and restored ponds across the colonisation process. The abundance-occupancy relationship was also compared between both sub-orders and pond age. Finally, the composition of odonate communities was investigated in relation to landscape context, structural connectivity and vegetation structure across the colonisation process.

## 2. Material and methods

### 2.1. Study sites

The study was led in Normandy, in north-western France. With 112 inhabitants per km<sup>2</sup>, this region is moderately populated and covers 5% of the country surface (data from 2016; [www.insee.fr](http://www.insee.fr)). The main landscapes are mostly shaped by low rolling hills, farmland, pastures, woodland and fragmented by small cities and transport infrastructures. Since restoration or creation works did not occur on the same year for all ponds, the study was carried out on three years (2017, 2018 and 2019) for 12 ponds and on two years (2018 and 2019) for 8 other ponds (Fig. 1.1). At the beginning of the survey, all ponds had been just created or restored after terrestrialization and complete drying out. Therefore, no dragonfly larvae or aquatic vegetation was present on the ponds upon digging completion for both creation and restoration works (*i.e.* “year zero”). Inventories of larvae started systematically on the next spring (subsequently denoted by “the first year”), after colonisation by odonates. In forest ponds, restoration works are often done before the pond dries completely out. Hence, no restored forest pond met the criteria of complete drying out for this study and only newly created ponds were sampled in forest context. In other landscape contexts (*i.e.* agricultural and suburban), ponds were either newly created (5 ponds) or restored (8 ponds; Table 1.1 Fig. 1.1).



**Fig. 1.1.** Map of the Normandy region indicating the location of the newly created ponds used in this study. Black squares show the main cities, grey dots indicate ponds that were sampled on three consecutive years (2017-2019) and white dots indicate ponds that were sampled on two consecutive years (2018-2019). Restored ponds are represented by a cross on the circle and created pond are represented by empty circles.

**Table 1.1.** Characteristics of the 20 ponds sampled on 2 or 3 years in Normandy, France. Pond ID refers to the code displayed on the map presented on Fig. 1.1.

Pond ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Presence of fish	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Tree cover in a 0 - 200 m buffer (%)	5	0	3	7	36	27	100	91	2	100	100	98	97	28	14	17	12	69	19	43
Pond shading (%)	0	0	30	0	20	0	80	50	10	50	50	50	50	40	20	80	30	0	10	40
Pond size (m <sup>2</sup> )	350	400	350	150	230	300	50	250	700	50	50	50	50	200	50	50	500	200	120	400
Artificial surfaces in a 0 - 200 m buffer (%)	10	0	18	14	19	38	0	5	6	0	0	0	0	4	3	5	15	0	14	13
Pond density in a 0 - 500 m buffer (nb./km <sup>-2</sup> )	0.0	0.0	2.5	2.5	1.3	0.0	1.3	2.5	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	1.3	3.8	5.1	0.7
Pond density in a 500 m - 1 km buffer (nb./km <sup>-2</sup> )	0.0	0.0	0.9	0.0	1.3	0.0	1.3	1.3	0.4	0.0	0.0	0.9	0.8	0.4	0.0	0.4	0.0	2.1	1.3	2.1
Pond density in a 1 km - 2 km buffer (nb./km <sup>-2</sup> )	0.0	0.0	0.3	0.4	0.3	0.4	0.3	0.3	1.1	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.1	0.1	0.0	0.3
Type of work (C : creation, R : restoration)	C	C	R	C	R	C	C	C	R	C	C	C	C	R	R	R	R	C	C	R
Monitoring duration (years)	3	3	3	3	3	2	3	3	2	3	3	2	2	2	2	3	3	3	2	2

## 2.2. Sampling method

All sites were visited between 12 April and 31 May in 2017, 2018 and 2019. On each year, a cartography of the vegetation of the ponds (*i.e.* aquatic and bank) was done based on the habitat classes used in the PLOCH and IBEM methods (Oertli et al. 2005; Indermuehle et al. 2010). The number of samples was determined according to the surface of the pond ( $A$ ) with the following formula  $n = 15.5 - 10.5 * \log_{10}(A) + 2.7 * (\log_{10}(A))^2$  given by Indermuehle et al. (2010) in the IBEM protocol. Between 5 and 7 samples were therefore taken on each pond during 2 or 3 years yielding to a total number of 295 samples collected for this study. Sampling points were distributed among the habitats in proportion to their coverage with at least 2/3 of the samples taken at the land-water interface, because this part of the pond was shown to host more macro-invertebrates (Indermuehle et al. 2010). In each sampled aquatic habitat, a 25 × 25 cm kick-net with a 0.5 mm mesh size was used to make broad eight-shaped movements during 30 seconds. Content of the net was then visually sorted in a tray filled with about 1 cm of water. All odonate larvae were collected and preserved in 80% ethanol. A precise determination was done under a Leica M205C Stereomicroscope using several identification keys (Müller 1990; Norling & Sahlén 1997; Heidemann & Seidenbusch 2002; Brochard et al. 2012). All specimens, including very small instars, were identified to the species level except for the genus *Sympetrum*, in which most individuals were too young. *A. imperator* and *A. parthenope*, *C. puella* and *C. pulchellum* were also grouped in *Anax* sp. and *Coenagrion* gr. *puella* respectively because they were difficult to distinguish at the larval stage.

## 2.3. Environmental variables of ponds

To describe the environmental characteristics of each pond, 8 variables were recorded (Table 1.1). At each visit, the global shading was visually estimated (*i.e.* proximity and height of trees in the southern part of the pond) and the presence of fish was reported. The software Qgis (QGIS Development Team, 2015) was used to measure pond area and calculate the percentage of area covered with trees and artificial surfaces (*e.g.* buildings, roads, bare lands) within a radius of 200 meters. Structural connectivity was assessed by recording the density of surrounding ponds within 3 buffer ranges: 0 – 500 m, 500 m – 1 km and 1 km – 2 km. Based on a database from the regional pond network (data from 2019; [www.pramnormandie.com](http://www.pramnormandie.com)) combined with aerial photographs, ponds that seemed favourable to a large richness in odonate species were identified. To simplify the selection and assessment based on aerial photographs, ponds whose size was < 50 m<sup>2</sup> were not considered.

Then, all the ponds containing least a few macrophytes (*e.g.* cattle tanks were excluded), and not completely shaded by large trees were then retained as favourable for odonates. No lake was present in the surrounding buffers. Large ditches presenting a rich vegetation structure were also recorded as potential sites for odonates, but lotic waterbodies like streams and rivers (*i.e.* La Seine) were not taken into account.

#### 2.4. Computation of community parameters

Since the number of samples differed according to pond size, a higher number of detected species was expected in ponds with more samples. To take this detection probability into account, a capture-recapture approach (Boulinier et al. 1998; Nichols et al. 1998) on presence-absence data was used in order to estimate species richness on each pond. Species richness (N) and detection probabilities (P) were estimated using the jackknife estimator associated with model M(h) (Burnham & Overton 1978; 1979; Otis et al. 1978), which assumes variability in detection probability among species within a pond, and among ponds and years. Extinction probabilities (PHI) and colonisation (B) between 2017 and 2018, and between 2018 and 2019, were calculated according to Nichols et al. (1998). The software ComDyn4 ([www.mbr-pwrc.usgs.gov/software/comdyn4.html](http://www.mbr-pwrc.usgs.gov/software/comdyn4.html)) was used to provide these parameters of community dynamics and detection probabilities for each pond between sampling years (Hines et al. 1999). The provided estimates N and B computed for the suborders Anisoptera and Zygoptera separately, and for all odonates together were used for further analyses. The number of extinct species (E) between two consecutive years was also calculated by multiplying the initial number of species detected during the sampling session on the first year ( $N_1$ ) by the local extinction probability (PHI) given by ComDyn4 between the two considered years.

## 2.5. Data analysis

All statistical analyses were performed using R software (version 3.6.2; R Core Team, 2019). Means are given  $\pm$  SD and model estimates are given  $\pm$  SE. No larva was found on pond 6 (Table 1.1) on both sampling years, although some adults of several species were observed during summer 2017. Therefore, the 12 samples from this pond were excluded from the analyses.

A Principal Component Analysis (PCA) was performed on the 7 continuous environmental variables characterizing the ponds (Table 1.1) to extract the Principal Components (PCs) of the dataset. The categorical factor for the presence of fishes was also included as a supplementary variable in the analysis. The two first Principal Components were used for further analyses. They explained respectively 39.6 % and 21.1 % of the variance of the PCA. The PC1 was negatively correlated with the surrounding tree cover in the 0-200 m buffer and pond shade, and positively correlated with pond size and surrounding artificial surface cover in the 0-200 m buffer (*i.e.* Pearson's correlation: all factors loadings  $> 0.7$ ; Table 1.2). This axis was therefore interpreted as a gradient in landscape openness, *i.e.* from smaller forest ponds to larger ponds in open anthropized landscapes. The presence of fishes was also positively related to PC1 (Spearman's correlation test:  $r = 0.53$ ,  $P = 0.02$ ). The PC2 was positively correlated with pond density in the 0-500m buffer and in the 500m-1km buffer (*i.e.* factor loadings respectively 0.87 and 0.68). This second axis was therefore interpreted as the gradient of structural connectivity in the surroundings of the ponds.

**Table 1.2.** Loading of each variable in the two principal components of the PCA. Correlation coefficients between variable and principal components that were  $> 0.7$  are printed in bold.

Environmental variable	PC1 Landscape openness gradient	PC2 Structural connectivity
Trees cover in a 0 - 200 m buffer (%)	<b>-0.867</b>	0.026
Pond shading (%)	<b>-0.726</b>	-0.056
Pond size (m <sup>2</sup> )	<b>0.793</b>	-0.316
Artificial surfaces in a 0 - 200 m buffer (%)	<b>0.711</b>	0.349
Pond density in a 0 - 500 m buffer (nb./km <sup>-2</sup> )	0.171	<b>0.873</b>
Pond density in a 500 m - 1 km buffer (nb./km <sup>-2</sup> )	-0.087	0.676
Pond density in a 1 km - 2 km buffer (nb./km <sup>-2</sup> )	0.569	-0.186
Variance of the PCA explained	39.6 %	21.1 %

Aquatic habitats of the PLOCH protocol (Oertli et al. 2005) were grouped into 3 main categories for the analyses: no vegetation, helophytes and hydrophytes. The helophytes were the most represented aquatic habitats on the ponds surveyed with 147 out of the 283 samples collected in this vegetation class during the three-year survey. Habitats without aquatic vegetation (*e.g.* bare bank, tree roots or dead leaves) represented 96 samples and hydrophytes represented 40 samples. The relationships between the presence of each aquatic habitat on the pond, the two PCs and age were tested using a binomial Global Linear Mixed Model (GLMM) performed using the R package “glmmTMB” (Magnusson et al. 2017). The pond was set as random effect to account for temporal pseudo-replication during this three-year survey. In all these models, vegetation structure in ponds did neither depend on the age of the pond nor on the environmental variables (all model  $P > 0.05$ ). This variable could therefore be used independently in subsequent analyses.

The effect of the type of works (*i.e.* creation or restoration) and pond age on the log-transformed estimated community parameters (*i.e.* N, E and B) was tested using Linear Mixed Models (LMM) with a dataset containing all 12 non-forest ponds, since all forest ponds sampled were created. The full dataset containing the 19 ponds was then used to investigate the relationships between community parameters, the two main PCs and pond age using LMM. All variables (*i.e.* PCs and age) were tested for additions and interactions in the models. These analyses were done for all odonates and for each suborder separately using the R package “glmmTMB”, with the pond as random effect to account for temporal pseudo-replication during this three-year survey.

For each year, the relationship between species abundance and species occupancy (*i.e.* proportion of ponds in which they were found) was investigated in both suborders (*i.e.* Anisoptera and Zygoptera) using analyses of covariance (ANCOVA). The ecological niche of species was defined by projecting species occurrences on each pond on the PC<sub>1</sub> (*i.e.* gradient in landscape openness). The mean value of species on PC<sub>1</sub> was then used to define forest specialists (*i.e.* negative value) and open landscape specialists (*i.e.* positive value). Generalist species were defined as species with a large niche breadth (*i.e.* amplitude on PC<sub>1</sub>  $> 3$ , which is more than two times the mean amplitude) and species occurring in no more than two ponds were categorized as rare species. Other species were categorized as intermediary (*i.e.* mean value ca. 0 and niche breadth  $< 3$ ). The occurrence and abundance of each ecological group of species and each sub-order was then modelled against the two PCs, age of the pond and aquatic habitats using GLMM following the Hurdle formulation (Brooks et al. 2017). This formulation works in two stages and was chosen because it allows to handle zero inflated abundance data. The first stage tests the presence or absence of the considered species

in each sample under a binomial regression (*i.e.* zero-inflated model). Then, the second stage works with the mean abundance in the samples where the species were present using a truncated negative binomial regression (*i.e.* conditional model). All variables were also tested for addition in the model but due to the limited size of the dataset, the interaction between the PCS and age could only be tested for abundance of odonates and for abundance of each suborder.

In all models, abundance was ln-transformed. Explanatory variables (*i.e.* PCs and age) were centred and standardised (Schielzeth 2010) to optimize interpretability of the estimates. Model selection was then based on the Aikake Information Criterion (AIC). When several models had a  $\Delta\text{AIC} < 2$ , only the model with the smallest number of significant variables was kept for interpretation. Pairwise post-hoc tests were performed with the Tukey p-value adjustment on the least-square means of estimates using the R package “lsmeans” (Lenth 2016).

## 3. Results

### 3.1. Description of the samples

A total of 4232 larvae was collected during the three years with a mean of  $17.3 \pm 28.7$  individuals and  $2.39 \pm 1.98$  species per sample. When including the two *Coenagrion* species (*i.e.* *C. puella* and *C. pulchellum*) and the two *Sympetrum* species (*i.e.* *S. sanguineum* and *C. striolatum*) which were pooled for the analyses, a total of 24 species was detected in this study, including 15 Anisoptera and 9 Zygoptera (*n.b.* no larvae of *A. parthenope* was found; Table 1.3). Only 2.6 % of the larvae could not be assigned to any taxon. They all belonged to the suborder Zygoptera and were recorded as “unidentified Zygoptera”. Observed species richness ranged from 0 on four sampling occasions (pond 7 in 2017 and 2018, pond 2 in 2019, and pond 17 in 2018) to 13 species for one sampling occasion on the pond 18 in year 2019. The mean probability of detection was  $0.84 \pm 0.18$  and only 6 sampling occasions out of 50 had a probability  $< 0.70$ . The probability of detection was similar for both suborders with a mean on all studied years of  $0.88 \pm 0.15$  in Anisoptera and  $0.86 \pm 0.18$  in Zygoptera. The most common taxa were *C. gr. puella*, *L. depressa* and *Sympetrum* sp.. The species *A. affinis*, *C. erythrea*, *L. fulva* and *O. brunneum* were found on only one occasion over the 3 years (Table 1.3).

**Table 1.3.** Total abundance of larvae for each taxon and number of ponds in which each species was detected over all studied years.

Species	Abundance	Number of Ponds
<b>Anisoptera</b>	<b>1386</b>	<b>19</b>
<i>Aeshna affinis</i>	1	1
<i>Aeshna cyanea</i>	209	11
<i>Anax</i> sp.	133	8
<i>Brachytron pratense</i>	1	1
<i>Cordulia aenea</i>	2	2
<i>Cordulegaster boltonii</i>	12	2
<i>Crocothemis erythrea</i>	2	1
<i>Libellula depressa</i>	537	13
<i>Libellula fulva</i>	1	1
<i>Libellula quadrimaculata</i>	9	2
<i>Orthetrum brunneum</i>	2	1
<i>Orthetrum cancellatum</i>	35	5
<i>Orthetrum coerulescens</i>	10	2
<i>Sympetrum</i> sp. <sup>a</sup>	432	13
<b>Zygoptera</b>	<b>2846</b>	<b>18</b>
<i>Coenagrion</i> gr. <i>puella</i> <sup>b</sup>	1102	15
<i>Coenagrion scitulum</i>	312	10
<i>Ceriagrion tenellum</i>	74	2
<i>Chalcolestes viridis</i>	214	6
<i>Enallagma cyathigerum</i>	99	4
<i>Ischnura elegans</i>	613	11
<i>Ischnura pumilio</i>	15	4
<i>Pyrrosoma nymphula</i>	305	8
Unidentified Zygoptera	112	11
<b>Total</b>	<b>4232</b>	<b>-</b>

<sup>a</sup> including the species *S. sanguineum* and *S. striolatum*

<sup>b</sup> including the species *C. puella* and *C. pulchellum*

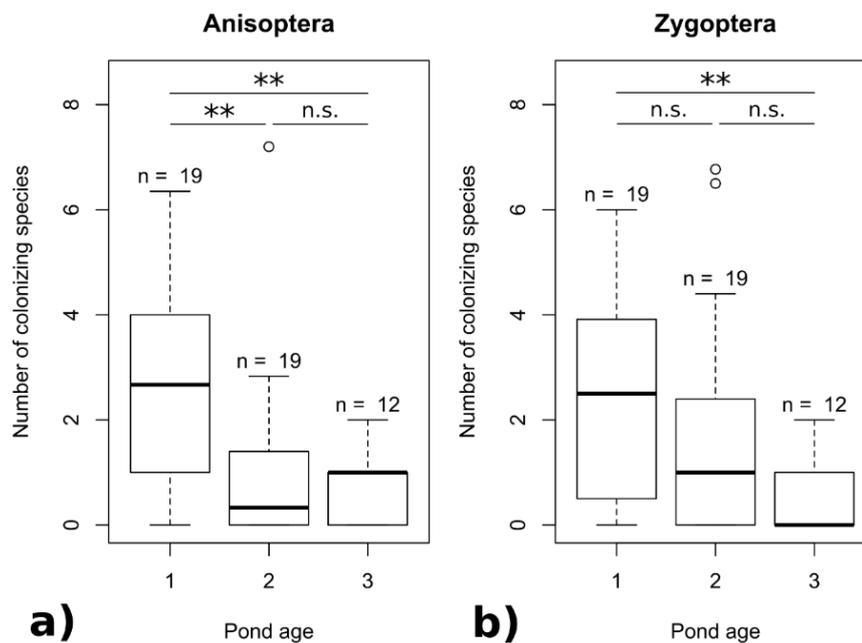
### 3.2. Colonisation and community dynamics

The type of work (i.e. creation or restoration) had no significant effect on the community parameters N (estimated species richness), B (colonisation) and E (extinction) for all odonates and for each suborder separately (p-values > 0.05 for the factor “creation or restoration” in all models).

Estimated species richness (N) of all odonates was not related to any explanatory variable. Estimated species richness of Anisoptera was positively related only to PC2 (i.e. gradient of structural connectivity) and estimated species richness of Zygoptera was positively related only to PC1 (i.e. gradient in landscape openness; Table 1.4). Mean values of N were  $2.64 \pm 1.94$  and  $2.75 \pm 2.48$  species for Anisoptera and Zygoptera respectively (Table 1.S1). The number of extinct species (E) was not related to any explanatory variable for total odonates and Anisoptera, but positively related to PC1 (i.e. gradient in landscape openness) for Zygoptera (Table 1.4). Mean values of E were  $0.46 \pm 0.64$  and  $0.21 \pm 0.47$  species for Anisoptera and Zygoptera, respectively. The number of colonising species (B) decreased significantly with the age of the pond for all odonates as well as for the two suborders studied separately (Table 1.4). For Zygoptera, colonisation was also positively related to PC1 (i.e. gradient in landscape openness) and negatively related to the age of the pond and the interaction between PC1 and the age of the pond. Colonisation occurred essentially during the first year, with a mean estimated number of  $2.63 \pm 1.86$  anisopteran species and  $2.27 \pm 1.96$  zygopteran species found on the first inventory (Fig. 1.2). On subsequent years, colonisation differed between both suborders. For Anisoptera, the mean number of colonising species decreased significantly between the first year ( $2.63 \pm 1.86$  species) and the second year ( $1.07 \pm 1.77$  species) and stayed thereafter significantly constant the third year ( $0.79 \pm 0.69$  species; Tukey post-hoc test, all  $P < 0.01$ ; Fig. 1.2a). For Zygoptera, the mean number of colonising species was not different between the first year ( $2.27 \pm 1.96$ ) and the second year ( $1.67 \pm 2.16$  species; Tukey post-hoc test,  $P = 0.358$ ), but was significantly lower on the third year ( $0.49 \pm 0.77$  species) compared to the first year (Tukey post-hoc test,  $P = 0.007$ ; Fig. 1.2b).

**Table 1.4.** Best Generalized Linear Mixed Models on the relationships between community parameters (log transformed), and pond characteristics. Columns PC<sub>1</sub> and PC<sub>2</sub> are the principal components extracted from the PCA analysis and age is the number of elapsed years since pond creation. Ponds (n=19) were included as random effects in the models. Estimates are displayed  $\pm$  SE and printed in bold when significant (i.e.  $P < 0.05$ ).

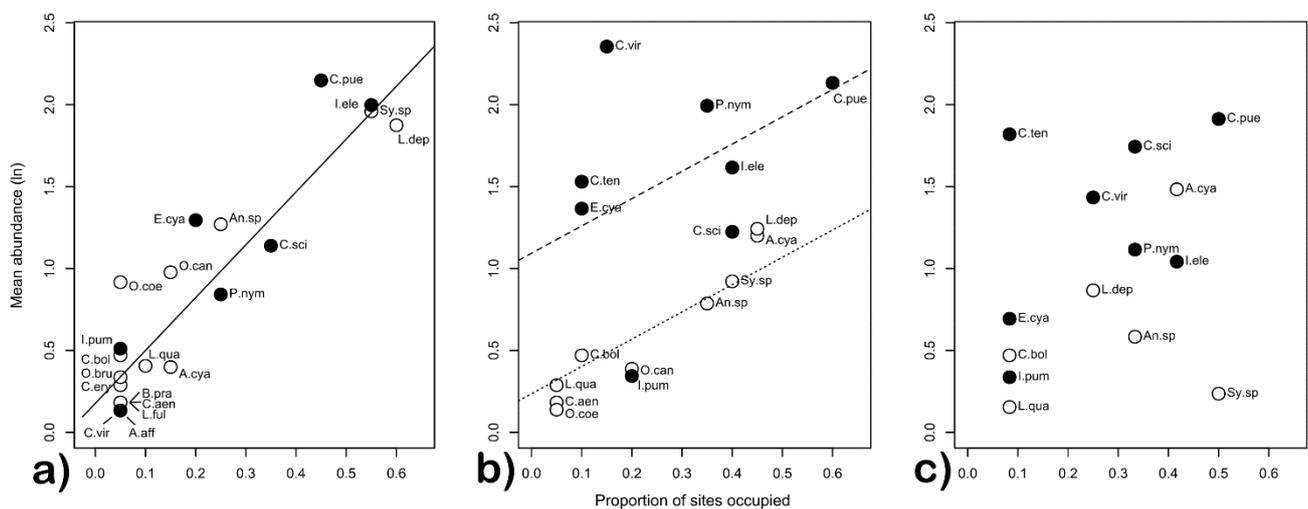
Parameter	PC <sub>1</sub>		PC <sub>2</sub>		Age		Interaction	
	estimate	P	estimate	P	estimate	P	estimate	P
Species richness (N)								
N all odonates	-	-	-	-	-	-	-	-
N Anisoptera	-	-	<b>0.21 <math>\pm</math> 0.06</b>	<b>0.004</b>	-	-	-	-
N Zygoptera	<b>0.18 <math>\pm</math> 0.07</b>	<b>0.018</b>	-	-	-	-	-	-
Extinction (E)								
E all odonates	-	-	-	-	-	-	-	-
E Anisoptera	-	-	-	-	-	-	-	-
E Zygoptera	<b>0.07 <math>\pm</math> 0.03</b>	<b>0.021</b>	-	-	-	-	-	-
Colonisation (B)								
B all odonates	-	-	-	-	<b>-0.52 <math>\pm</math> 0.14</b>	<b>&lt; 0.000</b>	-	-
B Anisoptera	-	-	-	-	<b>-0.34 <math>\pm</math> 0.11</b>	<b>0.003</b>	-	-
B Zygoptera	<b>0.44 <math>\pm</math> 0.13</b>	<b>0.004</b>	-	-	<b>-0.33 <math>\pm</math> 0.11</b>	<b>0.005</b>	<b>-0.17 <math>\pm</math> 0.07</b>	<b>0.014</b>



**Fig. 1.2** Number of estimated colonising species of Anisoptera (a) and Zygoptera (b) arriving on the pond for each age of the ponds. Number displayed above the boxes indicate the number of ponds studied for each year; \*\* indicates that the post-hoc  $p$ -value was  $< 0.01$  and n.s. indicates not significant relationship.

### 3.3. Abundance-occupancy relationships and community composition

On the first year after pond creation or restoration, the relationship between abundance and occupancy was significantly positive ( $F = 108$ ,  $P < 0.001$ ) and no difference was found between anisopteran and zygopteran species ( $F = 0.574$ ,  $P = 0.459$ ; Fig. 1.3a). On the second year, the relationship between abundance and occupancy was still significantly positive ( $F = 10.8$ ,  $P = 0.006$ ; Fig. 1.3b), but for a same proportion of sites occupied, the abundance of larvae was significantly higher for Zygoptera than for Anisoptera ( $F = 14.9$ ,  $P = 0.002$ ; Fig. 1.3b). On the third year, the relationship between abundance and occupancy was no longer significant ( $F = 2.03$ ,  $P = 0.182$ ; Fig. 1.3c), but Zygoptera were still significantly more abundant than Anisoptera for a same proportion of sites occupied ( $F = 6.69$ ,  $P = 0.036$ ; Fig. 1.3c).



**Fig. 1.3.** Relationships between abundance ( $\ln$ -transformed mean number of individuals per sample for each pond) and occupancy (proportion of occupied ponds) for (a) 19 ponds inventoried on their first year ( $R^2 = 0.84$ ), (b) 19 ponds inventoried on their second year; ( $R^2 = 0.59$ ) and (c) 12 ponds inventoried on their third year ( $r^2 = 0.31$ ). Open dots represent anisopteran species, filled dots represent zygopteran species. Regression lines are continuous for both suborders together, dotted for Anisoptera and dashed for Zygoptera.

The classification of the species based on their ecological niches gave 2 forest specialists, 3 generalists, 6 specialists of open landscapes and 10 rare species (Table 1.5). The species *Chalcolestes viridis* could not be attributed to any of these groups due to its intermediate niche position. The models of occurrences in the ponds showed contrasted responses to explanatory variables depending on the study group. The occurrence of all odonates was not related to any variable. The occurrences of all Anisoptera and Zygoptera were significantly related to neither PC<sub>1</sub> (*i.e.* gradient in landscape

openness) nor PC2 (*i.e.* gradient of structural connectivity; all  $P > 0.05$ ; Table 1.5a). This global non-significance can be explained for PC1 by the fact that, specialists of open landscapes are positively related to this axis whereas forest specialists are negatively (Table 1.6a). However, there was a significant positive effect of PC2 on the occurrence of generalist species and specialists of open landscapes (Table 1.6a). Age of the pond had a significant negative effect on the occurrence of Anisoptera, but not Zygoptera. The occurrence of generalists also tended to be positively related to pond age, whereas the occurrence of forest specialists was significantly negatively related to pond age (Table 1.6a). The vegetation structure had no effect on the occurrence of Anisoptera, but a significant positive effect on the occurrence of Zygoptera and specialists of open landscapes. Especially, samples collected in helophytes were significantly more likely to contain larvae of these groups than samples collected at places without vegetation (Table 1.6a).

There was no significant relationship between total abundance of all odonates, Anisoptera or Zygoptera and PC1 (Table 1.6b). This global non-significance can be explained by the fact that the abundance of forest specialists was significantly negatively related to PC1 and the abundance of specialist species of open landscapes was significantly positively related to PC1. The total abundance of odonates was significantly positively related to PC2 (Table 1.6b). This positive relationship was also found between Zygoptera, generalists, specialists of open landscapes and PC2. Pond age tended to have a negative effect on total abundance of Anisoptera larvae (Table 1.6b). Pond age also had a significant negative effect on the abundance of generalist species (Table 1.6b). The vegetation structure had no significant effect on the groups tested, although this variable improved the model AIC for the abundance of total odonates.

**Table 1.5.** Categories of ecological niches for each species and representation on a presence-absence matrix. Ecological niches were defined based on the mean value and standard deviation of species occurrences projected on PC<sub>1</sub> (i.e. gradient in landscape openness). Generalist species are denoted “gen”, specialists are denoted “spe” and the intermediate species are denoted “int”. Species and ponds are ranked according to their position on PC<sub>1</sub>.

Genus species	Mean value on PC <sub>1</sub>	Breadth	Ecological niche	Pond number																Sum		
				7	12	10	11	13	16	8	14	18	15	20	19	2	5	1	17		3	4
<i>Cordulegaster boltonii</i>	-1.29	-	rare			■		■														2
<i>Cordulia aenea</i>	-0.99	-	rare					■	■													2
<i>Brachytron pratense</i>	-0.72	-	rare																			1
<i>Libellula fulva</i>	-0.72	-	rare																			1
<i>Pyrhosoma nymphula</i>	-0.72	1.43	forest spe		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	8
<i>Aeshna cyanea</i>	-0.68	1.78	forest spe	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	11
<i>Orthetrum coerulescens</i>	-0.11	-	rare																			2
<i>Crocothemis erythraea</i>	-0.03	-	rare																			1
<i>Coenagrion gr. puella</i>	0.07	3.22	gen	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	15
<i>Chalcolestes viridis</i>	0.09	1.70	int																			6
<i>Libellula quadrimaculata</i>	0.11	-	rare																			2
<i>Libellula depressa</i>	0.20	3.22	gen	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	13
<i>Ceriagrion tenellum</i>	0.42	-	rare																			2
<i>Sympetrum sp.</i>	0.42	3.20	gen				■		■	■	■	■	■	■	■	■	■	■	■	■	■	12
<i>Orthetrum brunneum</i>	0.48	-	rare																			1
<i>Anax sp.</i>	0.49	1.52	open spe							■		■										8
<i>Orthetrum cancellatum</i>	0.51	1.23	open spe																			4
<i>Enallagma cyathigerum</i>	0.68	1.27	open spe																			4
<i>Coenagrion scitulum</i>	0.69	2.08	open spe																			10
<i>Ischnura pumilio</i>	0.69	0.56	open spe																			4
<i>Ischnura elegans</i>	0.70	2.08	open spe																			11
<i>Aeshna affinis</i>	0.98	-	rare																			1
Mean value	0.06	1.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2

**Table 1.6.** Best Generalized Linear Mixed models on the relationships between odonate occurrences in the samples (a), species abundance on the ponds on which the species were present (b), and ponds characteristics. Columns PC1 and PC2 are the principal components extracted from the PCA analysis; age is the number of elapsed years since pond creation; vegetation structure corresponds to the aquatic habitat in which the sample was taken. Pond ( $n=19$ ) were included as random effects in the models. Estimates are displayed  $\pm$  SE and printed in bold when significant (i.e.  $P < 0.05$ ). For the vegetation structure the category “no vegetation” (i.e. “NV”) was used as reference, “He.” stands for helophytes and “Hy.” stands for hydrophytes. The post-hoc  $p$ -values were computed on the least square means for each combination of vegetation structure category.

## a) Occurrences (Zero-inflated model)

Species	PC1		PC2		Age		Vegetation structure		
	estimate	<i>P</i>	estimate	<i>P</i>	estimate	<i>P</i>	He.	Hy.	<i>P</i> (Post-hoc) NV-He; NV-Hy; He-Hy
Total Odonata	-	-	-	-	-	-	-	-	-
Anisoptera	-	-	-	-	<b>-0.8 <math>\pm</math> 0.3</b>	<b>0.019</b>	-	-	-
Zygoptera	-	-	-	-	-	-	<b>1.4 <math>\pm</math> 0.6</b>	0.7 $\pm$ 0.6	<b>0.043</b> ; 0.514; 0.578
Forest specialists	<b>-3.2 <math>\pm</math> 1.6</b>	<b>0.012</b>	-	-	<b>1.2 <math>\pm</math> 0.9</b>	<b>0.010</b>	-	-	-
Generalists	-	-	<b>1.4 <math>\pm</math> 0.6</b>	<b>0.017</b>	-0.6 $\pm$ 0.3	0.051	1.2 $\pm$ 0.6	-0.4 $\pm$ 0.7	0.123; 0.851; 0.079
Open specialists	<b>2.7 <math>\pm</math> 0.8</b>	<b>0.001</b>	<b>1.8 <math>\pm</math> 0.6</b>	<b>0.002</b>	-	-	<b>2.5 <math>\pm</math> 0.8</b>	1.9 $\pm$ 0.9	<b>0.010</b> ; 0.104; 0.736
Rare species	-	-	-	-	-	-	-	-	-

## b) Abundance (Conditional model)

Species	PC1		PC2		Age		Vegetation structure		
	estimate	<i>P</i>	estimate	<i>P</i>	estimate	<i>P</i>	He.	Hy.	<i>P</i> (Post-hoc) NV-He; NV-Hy; He-Hy
Total Odonata	-	-	<b>0.5 <math>\pm</math> 0.2</b>	<b>0.013</b>	-	-	0.5 $\pm$ 0.2	0.2 $\pm$ 0.3	0.104; 0.790; 0.508
Anisoptera	-	-	-	-	-0.4 $\pm$ 0.2	0.061	-	-	-
Zygoptera	-	-	<b>0.5 <math>\pm</math> 0.2</b>	<b>0.022</b>	-	-	-	-	-
Forest specialists	<b>-0.7 <math>\pm</math> 0.4</b>	<b>0.042</b>	-	-	-	-	-	-	-
Generalists	-	-	<b>0.6 <math>\pm</math> 0.30</b>	<b>0.023</b>	<b>-0.3 <math>\pm</math> 0.2</b>	<b>0.022</b>	-	-	-
Open specialists	<b>1.4 <math>\pm</math> 0.4</b>	<b>0.000</b>	<b>1.0 <math>\pm</math> 0.2</b>	<b>0.000</b>	-	-	-	-	-
Rare species	-	-	-	-	-	-	-	-	-

## 4. Discussion

From their first two or three years after creation or restoration, studied ponds hosted a large panel of odonate species. The 24 species recorded during this survey represent more than 45 % of the regional autochthonous odonate species (*i.e.* 52 species; [www.biodiversite.normandie.fr](http://www.biodiversite.normandie.fr)). This proportion is slightly higher than in previous studies on ponds in southern France (*i.e.* 40%; Ruggiero et al. 2008) or constructed wetlands in Finland (*i.e.* 40%; Huikkonen et al. 2019). It confirms that, even on the first years, these ponds are able to support a non-negligible part of the regional pool of species.

Odonates showed a high colonisation rate on the first year after pond creation or restoration compared to subsequent years. However, the patterns of colonisation were different between the two suborders. For Anisoptera, most colonising species arrived on the first year and very few on subsequent years, whereas in Zygoptera, many species also arrived on the second year. This difference of colonisation pattern might reflect a difference in dispersal abilities between both suborders. The estimated total number of species and the number of extinctions did not differ according to pond age, showing that the turnover was constant among the years. Therefore, the colonisation rate seems to be the only varying community parameter over the first years. The group of generalist species (*i.e.* *C. puella*, *L. depressa* and *Sympetrum* sp.) presented higher occurrences and abundances at the beginning of the survey. These three species can be considered as pioneer species with affinity for early successional stages (Inden Lohmar 1997). On the contrary, the occurrence of forest specialists (*i.e.* *A. cyanea* and *P. nymphula*) increased with pond age. This may indicate that a turnover between pioneer and species of later successional stages could occur at least in forest ponds.

Sampling was conducted during the middle of springs, although Hill et al. (2016) proposed that odonate larvae should be sampled in autumn to maximised the observed biodiversity. However, in their study, Hill et al. (2016) found a mean of 1.6 odonate species per pond in autumn (Hill et al. 2016), which is about 4 times less than in the present study. Since most dragonfly species lay eggs during the summer, larvae are very small during following months. The identification of the 22 taxa of our study would probably have been too difficult with an autumnal sampling. Nevertheless, adding a second sampling session later in the summer as performed by Huikkonen et al. (2019), may be a good improvement to our method to allow better identification of late emerging species like

*Sympetrum* spp. and detection of *Sympecma fusca*, the only species overwintering at the adult stage in our region.

The IBEM protocol was designed to detect at least 70 % of the target invertebrates, *i.e.* coleoptera and gastropoda (Indermuehle et al. 2010). In this study, only 12 % of the sampling occasions were below this threshold, assessing that most species present in the ponds were detected during the survey. Adapting the number of samples to pond area enabled us to keep a high and homogeneous sampling grain on all sites. This sampling grain and spatial extent are the main drivers to assess species occupancy distribution and especially, condition the proportion of rare species detected (Korkeamäki et al. 2018). According to the positive species-area relationship, the number of rare species should increase with the scale of the study because samples taken from large areas are more likely to be heterogeneous than samples from smaller areas (McGeoch and Gaston 2002). In the present study of 230 km longitudinal extent and 150 km latitudinal extent, only 20 ponds were sampled, but the high sampling grain on ponds enabled us to detect ten rare species (*i.e.* 43 % of the total species pool) that were present in less than 10 % of the waterbodies. They were mostly located in the southern and western parts of the Normandy, which are the distribution range limits for several odonate species (CERCION, 2017). This proportion of rare species is comparable to a previous 900 km latitudinal study from Northern Europe in which 50% of the odonate species (*i.e.* larvae, exuviae, and adults) occurred in less 10% of the lakes and ponds (Korkeamäki et al. 2018). Five species were found only on one pond and only on the first year of the survey. They were all Anisoptera and their presence on ponds might be explained by the behaviour of few isolated females. To avoid sexual harassment during oviposition, females sometimes look for less competitive places in the surroundings of their mating site (Corbet 1999). For instance, the larvae of *L. fulva* and *B. pratense* that were found on the first year on a small and shaded pond (*i.e.* pond 16), may come from the population of a larger, sunny and well vegetated pond located only 140 m away. On subsequent years, new local competition may have prevented these vagrant females to come.

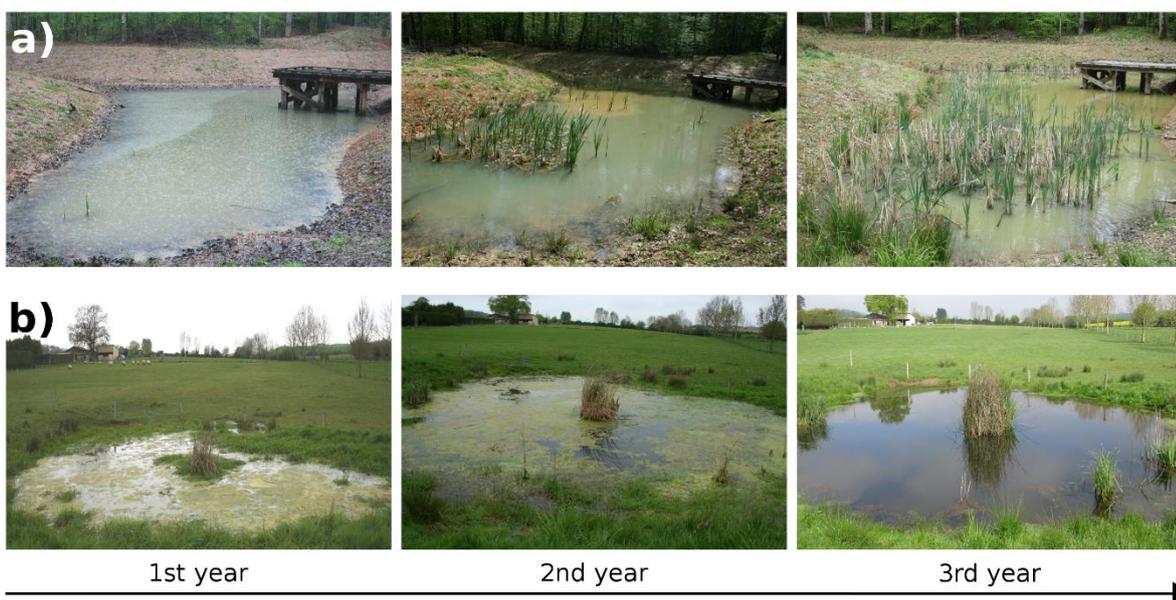
The colonisation process is usually characterized by a low occupancy and abundance on the first years (Gaston et al. 2000). Abundance and occupancy then increase conjointly with time as the species multiply and spread to new sites (Gaston et al. 2000). On the first year of this study, this pattern was very clear with a strong abundance-occupancy linear relationship and about half the species (*i.e.* 10/21 species) found only on one pond. On the second year, many species that were present on one pond only either disappeared or increased in abundance. The results showed that the abundance-occupancy relationship also become weaker and especially, because zygopteran species

increased in abundance and this, regardless of pond area. Zygoptera are smaller than Anisoptera and their metabolism has therefore lower energetic needs, which may enable them to be more numerous for the same amount of resources (Heino 2008). On the contrary, the frequent cannibalism among Anisopteran species (Suhling et al. 2015) probably implies density dependence effects and limit their abundance in small ponds. On the third year, the abundance of larvae was still higher in Zygoptera than in Anisoptera but we did not observe the relationship between abundance and occupancy.

The landscape context of a pond has therefore a marked effect on the composition of odonate communities (Le Gall et al. 2018). For instance, although it has no direct effect on growth and survival of larvae, an increase in canopy cover on ponds was shown to decrease both the number of visits by adults and the species richness (French & McCauley 2018). In our study, the effect of canopy cover was also associated with the size of the pond, suggesting that in the studied region, pond size is related to the landscape context and thus, to human activities. Most odonate species seemed to prefer large ponds in open landscapes, while some others were mostly present on smaller forest ponds. Many species were indeed categorized as specialists of open landscapes. In this group, abundance was also positively linked to the openness of the landscape and large pond size. Since odonates are ectothermic organisms, most species prefer open areas which enable a better body heating in temperate regions. Moreover, sun-exposed ponds often present a higher diversity in vegetation structures favouring a large panel of species (Oertli et al. 2002; Sayer et al. 2012). As expected, the species *A. cyanea* and *P. nymphula*, that are more specialised in forest environments (Goertzen & Suhling 2013; Le Gall et al. 2018) were more present in ponds with low values along the openness gradient. Interestingly, the pond containing the higher diversity (*i.e.* pond 18; mean estimated species of  $13.9 \pm 1.6$  on the 3-year survey) was located at the border of a meadow surrounded by a large forest. The interface between forest and open contexts, associated with the proximity of other ponds, probably enabled to maximise the species richness on this pond, providing favourable conditions for both forest and non-forest species.

The presence of fish is usually related to the size of the pond and only few occur in ponds smaller than 1000 m<sup>2</sup> (Søndergaard et al. 2005), excepted in urban areas where inhabitants often release goldfish (Copp et al. 2005). Fish impacts odonate communities by predation or changing the composition of the prey and vegetation structure (Wittwer et al. 2010). In our study, fish were detected in only 2 large ponds (*i.e.* pond 3 and 9) in an open landscape context. The species richness and abundance on these two ponds are probably lower than it could be expected given the size of this pond but it could not be tested in this study.

Vegetation is often the main factor driving odonate communities (Goertzen & Suhling 2013; Perron & Pick 2020). Especially, high heterogeneity in vegetation structures with floating and submerged macrophytes supports higher species richness and abundances (Raebel et al. 2012a; Huikkonen et al. 2019). In this study, vegetation was related significantly to the global occurrence of Zygoptera and the global occurrence of species specialist of open landscape contexts. However, it was not significantly related to the abundance of any ecological groups, which was unexpected because vegetation often provides hiding and roosting places for larvae (Huikkonen et al. 2019). During the process of pond colonisation, we could expect a gradual increase in the vegetation cover year after year. However, inventoried classes of vegetation structure did not significantly change according to the type of work (*i.e.* creation of restoration), pond age or environment. While some ponds like pond 8 showed a pattern of colonisation by aquatic vegetation from one year to another (Fig. 1.4a), others, like pond 4, were quickly colonised on the first year and then underwent regression due to exogenous perturbations (*e.g.* grazing; Fig. 1.4b). Previous studies showed that odonate were very sensible to grazing during summer, with a strong impact on larval abundance and diversity (Foote et al. 2005). Here, cattle pressure on agricultural ponds may have been responsible for the non-significant link between vegetation structure observed in spring and odonate occurrence and abundance.



**Fig. 1.4.** Illustration of changes in vegetation structure on pond 8 (a) and 4 (b) during the three first years following pond creation.

Ponds constitute a network of discrete habitats that can act as stepping stones to facilitate movements of freshwater organisms across a terrestrial landscape (Hassall, 2014). Odonate

communities usually present strong spatial autocorrelation within a distance up to 13 km (Briers & Biggs 2005). This spatial autocorrelation sometimes explains more variability in species composition than surrounding land use and within-habitat environment (Hamasaki et al. 2009). In our study, species richness of Anisoptera was positively related to the density of neighbouring ponds favourable to odonates, while it was only related to landscape context in Zygoptera. The structural connectivity was also the main factor that shaped all odonate abundance and especially, species of the suborder Zygoptera, specialists of open landscapes and generalists. This general positive relationship between connectivity and abundance probably results from a metapopulation process that enables to support larger populations when they are connected by frequent dispersal events (Hanski 1998; Heino et al. 2015). In our study, the response to structural connectivity may be more pronounced for Zygoptera because they have lower dispersal abilities (e.g. Conrad et al. 1999; Angelibert & Giani 2003) and need more proximity between their habitat niches. Maintaining a high connectivity between ponds may therefore support the maintenance of the populations of the smaller species and increase their viability at the landscape scale (Hanski & Ovaskinen 2000; Thornhill et al. 2018).

Over decadal-centennial timescales, natural ponds have always been ephemeral habitats that undergo successions of natural infilling over years (Sayer et al. 2012). Therefore, inhabiting aquatic species must be able to move regularly to find a place at an optimal successional stage for their development (Sayer et al. 2012; Janssens et al. 2018) and this, as long as connectivity between ponds allows it. Using data from open landscape context, no difference was found in species richness and colonisation dynamics between created and restored ponds suggesting that both type of works can contribute equally to enhance odonate diversity. At some created ponds (e.g. pond 4), plants like *Glyceria* sp. were able to colonise on the first year after pond digging, providing habitats for a large number of species and high abundances of odonates. On the contrary, some restored ponds (e.g. pond 5) were only slowly colonised by aquatic vegetation and hosted only few species with low abundance. Our result supports the idea that even during the first years after creation, new ponds are able to host a similar species richness as ponds restored after complete drying out. We therefore suggest that pond restoration should perhaps not always be preferred to pond creation in the freshwater conservation schemes.

Overall, odonates demonstrated capabilities to establish very quickly in ponds, with most species colonising from the first summer after impoundment. The landscape openness associated with pond size was the main gradient driving the occurrence of species. Abundances were strongly related to pond connectivity, especially for Zygoptera. Only occurrences of Zygoptera and specialists

of open landscapes were significantly also associated with vegetation structure in this study. Nevertheless, the establishment of vegetation that provides habitats for larvae is often likely influenced by grazing during summer. These features along with the influence of landscape context and connectivity should be emphasised in further studies on the conservation value of ponds. Our work showed that created ponds do not perform differently from restored ones in their abilities to host odonate biodiversity in open landscapes. Creating new ponds at strategic locations in the regional blueway network is a valuable mean to increase connectivity between ponds of high conservation value and support the maintenance of critical populations.

## **5. Acknowledgements**

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## 6. Supplementary material

**Table 1.S1.** Estimated species richness ( $N$ ) and standard error for each pond and each year of sampling.

Pond	2017		2018		2019	
	Estimate	SE	Estimate	SE	Estimate	SE
BEA	7.08	0.47	16.25	5.13	5.80	1.01
BER	2.00	0.00	13.22	5.25	0.00	0.00
BSD	7.60	1.43	0.00	0.00	2.00	0.00
CER	12.24	1.67	13.98	4.19	15.44	6.70
CHB	-	-	5.00	0.00	2.86	1.28
EC1	1.00	0.00	1.00	0.00	4.00	0.00
EC3	5.33	1.19	4.00	0.00	3.80	1.06
EC4	-	-	1.00	0.00	3.80	1.58
EC5	-	-	2.80	1.41	11.04	4.52
GUE	-	-	8.83	0.91	8.67	2.49
MAF	1.00	0.00	5.80	1.37	6.83	1.34
MAR	0.00	0.00	6.00	0.00	7.80	1.39
MBR	-	-	0.00	0.00	1.00	0.00
MC1	3.35	0.00	1.00	0.00	1.00	0.00
MC2	-	-	4.00	0.00	6.80	1.16
MEB	6.67	0.78	5.00	0.00	5.83	1.95
PIR	-	-	8.67	2.09	20.10	8.68
REP	-	-	0.00	0.00	0.00	0.00
SMV	5.19	0.38	6.83	2.55	5.67	3.29
SOB	8.33	2.94	5.67	1.47	4.67	1.50

## Part II

# Larval development, consequences on adult condition and natal dispersal

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## **Chapter 2**

### **Biometry of the large dragonfly *Anax imperator* (Odonata, Aeshnidae): A study of traits from larval development to adults**

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#### **Abstract**

Insect larval development affects adult traits but biometric relationships remain poorly understood, especially in large odonates. In this study, morphological trait measurements were done on larvae, exuviae and adults of *Anax imperator*. They were used to investigate the effects of early development on adult condition. Results showed an increase in larval length within the final instar, and exuviae were significantly longer than last instar larvae. Length and body mass of teneral were strongly related to the length of their exuviae. Adult males were significantly longer than adult females, while both had the same body mass at emergence. Length of teneral was negatively related to the date of emergence in both sexes. During maturation, males showed only a little gain, while females greatly increased in body mass. Mature specimens were also significantly longer than teneral ones. Body mass of mature males and length of mature females were both influenced by the date of capture. Wing length did not differ between sexes or latitudes. This study underscores the importance of taking into account the larval growth to better understand adult condition of odonates.

**Key words:** Adult condition, *Anax imperator*, Body length, Body mass, Larval rearing, Odonata, Sexual size dimorphism, Traits

## 1. Introduction

Phenotypic traits are widely used in plants and animals to investigate organismal performances, such as survival or mating success (McGill et al., 2006; Violle et al., 2007; Lailvaux & Husak, 2014). In the last few decades, trait-based approaches were extended to the levels of populations, communities and ecosystems (Enquist et al., 2015; Kremer et al., 2017). Multivariate analyses and complex models now enable to predict, among others, species or population distribution along environmental gradients (Litchman et al., 2010; Santos et al., 2017). Body size can be used as a proxy of organismal fitness (Blanckenhorn, 2000) and the Bergmann's Rule is widely used to link body size to both temperature and latitudinal gradient (Watt et al., 2010; Faurby & Araújo, 2017). Originally developed for warm-blooded animals, this ecological concept was then extended to many invertebrate taxa including insects (Honěk, 1993; Arnqvist et al., 1996; Chown & Gaston, 2010). In odonates, body size and other traits, such as voltinism (*i.e.* number of generations per year), are driven by temperature (Corbet et al., 2006; Zeuss et al., 2017). Latitudinal gradients were shown to have a significant impact on the size of eggs, larvae and adults for Zygoptera (De Block & Stoks, 2003; Johansson, 2003; Stoks & De Blok, 2011; Sniegula et al., 2016). Moreover, body mass and size also affect female fertility and male mating success of territorial species (Anholt et al., 1991; Grether, 1996; Serrano-Meneses et al., 2007; Samejima & Tsubaki, 2010). Usually, in odonates, individuals with a larger body appear to benefit a better general fitness (Sokolovska et al., 2000), but this trend is probably counterbalanced by stabilising selection (Thompson & Fincke, 2002). For instance, longer larval development necessary to produce larger adults also results in higher predation risks (Waller & Svensson, 2017). Previous studies focused mostly on Zygoptera and only little is known about selection pressures that constrain large dragonflies' traits from larval stages to the emergence of adults (Stoks & Córdoba-Aguilar, 2012).

In the cosmopolitan genus *Anax*, larvae measure up to 70 mm (Suhling et al., 2014) and are commonly found in high densities in ponds (Calvert, 1934; Corbet, 1955). They are easy to rear, making them a good model for predator behaviour and diet in ecological studies (Folsom & Collins, 1984; Bergelson, 1985; Blois, 1985; Blois & Cloarec, 1985; Blois-Heulin, 1990a & 1990b). They were also often used to investigate the effect of predators on prey traits and community assemblages (Stav et al., 2000; Stoks & McPeck, 2003; McCauley, 2005; Relyea, 2012; Klecka & Boukal, 2013). Adults are robust and males exhibit a territorial behaviour in several species (Corbet, 1999). The species *Anax imperator* Leach, 1815, is the largest dragonfly of occidental Europe and is very commonly found on large waterbodies with vegetation (Heidemann & Seidenbusch, 2002; Brochard et al., 2012). Its

distribution area ranges from South Africa to Sweden (Dijkstra, 2006; Tarboton & Tarboton, 2015). Comparisons between populations under different latitudinal contexts can be carried out, since no migration is known for this species (Corbet, 1999). Differences in voltinism were also reported. For instance, in Europe, southern populations are able to have two generations per year (*i.e.* bivoltines), while most northern populations need two years to complete a life cycle (*i.e.* semivoltines; Corbet, 2006). Aside from major works performed in England (Corbet, 1955; Corbet, 1957), little data is available on larval traits of this common species (Portmann, 1921; Robert, 1958; Serrano-Meneses et al., 2007; Lamelas-López et al., 2017). Studies encompassing the whole life cycle or considering relationships between larval and adult traits of large odonates would enable to better understand the carry-over effects related to larval development conditions on adult fitness (Stoks & Córdoba-Aguilar, 2012). Unfortunately, body size or mass data from adult *A. imperator* are scarce (but see Serrano-Meneses et al., 2007). Indeed, young imagines of this species are difficult to study, since they emerge mostly during the night and take flight at dawn (Corbet, 1957). After maturation adults fly very fast and stay often away from pond banks, a behaviour that makes them hard to catch. Nevertheless, identifying variations in larval development within and between voltinism classes and dispersal events of adults along latitudinal gradients could be a key to understand maintenance of populations and colonisation in different parts of its distribution range. For example, the migratory species *Anax junius* (Drury, 1773) is able to cover several hundreds of kilometers during its life time (Wikelsky et al., 2006), leading to competition between different cohorts of migrants and resident populations in some places (Crumrine, 2010).

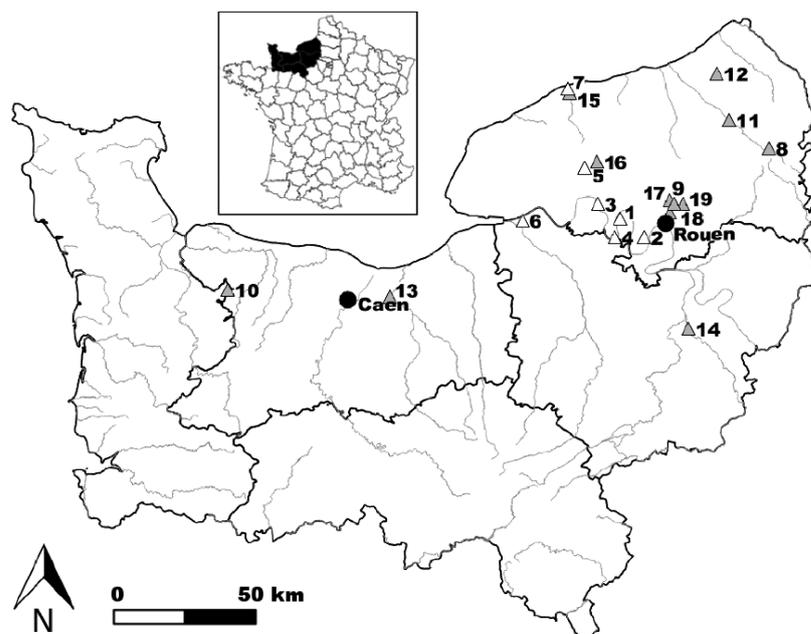
In the present context of increasing landscape fragmentation (Fardila et al., 2017), physical barriers to dispersal, such as main roads or cities, are obstacles to establishment, growth and *a posteriori* maintenance of isolated populations (Haddad et al., 2015). Moreover, dispersal is related to intrinsic traits of species that determine movement capacity. For instance, wingspan can be used as a proxy to discriminate dispersal abilities among butterfly species (Burke et al., 2011; Sekar, 2012; Slade et al., 2013). However, no relationship between wing shape and effective dispersal could be found in Trichoptera (Lancaster & Downes, 2017). No similar work on the relationship between traits and dispersal has been done yet for odonates, but the species' range could be correlated with wing size for several Zygoptera (Rundle et al., 2007; Swaegers et al., 2014). Besides these interspecific comparisons, only little evidence is known about the intraspecific effects of traits, such as wingspan or body size, on dispersal capacity (Taylor & Merriam, 1995; Conrad et al., 2002) and migration (Michiels & Dhont, 1989).

In this study, several growth traits between both life stages of the dragonfly *A. imperator* were investigated. Using measurements from larvae and exuviae, we first tested the effect of the preservation method and compared the larval growth between sexes and stadia, with emphasis on the metamorphosis occurring during the last larval instar. Then, reared teneral and mature adults were measured and the relationship between larval and adult traits was investigated. The effect of seasonality on adult traits was also studied to test the hypothesis that emerging adults are smaller at the end of the season for *A. imperator*. At each stage of life, males and females' traits were compared in order to investigate Sexual Size Dimorphism (SSD). Finally, the influence of latitude on body size of larvae and wing length of adults was discussed.

## 2. Material and methods

### 2.1. Sampling ponds and methods

Normandy region is located in north-western France and covers 5% of the country (Fig. 2.1). With 3.3 million inhabitants and a density of 112 inhabitants per km<sup>2</sup> (data from 2016; www.insee.fr), this area is moderately populated. Landscape is mostly shaped by farmlands, pastures, cities, sparse forests and low rolling hills. Nineteen ponds were selected according to their accessibility and ability to host *A. imperator* larvae (e.g. large and sunny ponds with abundant vegetation). A total of 345 larvae and 143 exuviae were collected in years 2014 and 2017 (Fig. 2.1; Table 2.1).



**Fig. 2.1.** Map of the study area in Normandy (France) with the location of the ponds sampled in 2014 (white triangles) and 2017 (grey triangles).

**Table 2.1.** Sampling ponds and counts of the different odonate stages sampled. ID refers to the numbers given on Fig. 2.1

ID	Year	Purpose	Site	Larvae	Exuviae	Adults	Coordinates WGS84 (N,E)
1	2014	Material stored	Anneville- Ambourville	31	3	0	49.458, 0.876
2	2014	Material stored	Mare Epinay	5	0	0	49.404, 0.987
3	2014	Material stored	ND-de-Bliquetuit	63	0	0	49.502, 0.770
4	2014	Material stored	Jumièges	65	15	0	49.402, 0.852
5	2014	Material stored	Valliquerville	2	1	0	49.609, 0.704
6	2014	Material stored	Marais Vernier	42	0	0	49.445 0.426
7	2014	Material stored	Veulettes sur mer	3	124	0	49.852, 0.609
8	2017	Material stored	Beaussault	11	0	0	49.682, 1.555
9	2017	Material stored	Métropole de Rouen (a)	7	0	0	49.480, 1.102
10	2017	Material stored	Forêt de Cerisy	7	0	0	49.199,-0.912
11	2017	Material stored	Mesnières en Bray	1	0	0	49.764, 1.366
12	2017	Material stored	St Ouen sous Bailly	4	0	0	49.905, 1.305
9	2017	Larvae reared	Métropole de Rouen (a)	44	0	0	49.480, 1.102
13	2017	Larvae reared	Bures sur Dives	1	0	0	49.200,-0.169
14	2017	Larvae reared	Heudreville	42	0	0	49.133, 1.197
15	2017	Larvae reared	Paluel	15	0	0	49.835, 0.625
16	2017	Larvae reared	Yvetot	2	0	0	49.629, 0.761
9	2017	Adult measurements	Métropole de Rouen (a)	0	0	27	49.480, 1.102
17	2017	Adult measurements	Métropole de Rouen (b)	0	0	19	49.484, 1.099
18	2017	Adult measurements	Métropole de Rouen (c)	0	0	4	49.469, 1.093
19	2017	Adult measurements	Métropole de Rouen (d)	0	0	1	49.481, 1.120
<b>TOTAL</b>				<b>345</b>	<b>143</b>	<b>51</b>	

Among all larvae, 211 were collected in 2014 from 7 ponds and 30 were collected in 2017 from 5 ponds during the period May-July and preserved in pure ethanol. Collection was made using a 25 × 25 cm kick net with a 0.5 mm mesh size. Between 11 May and 13 June 2017, 104 other larvae were collected from 5 ponds using 50 × 50 cm kick nets with 6 mm and 12 mm mesh sizes, and individually reared (Fig. 2.1; Table 2.1). Using nets with larger mesh size to collect last instar larvae enabled a more efficient collection targeting only larger material. In both years, larvae were sampled by drawing broad eight-shaped movements in the vegetation. Efforts were focused on floating macrophytes, such as *Potamogeton* sp. or *Ranunculus* sp., and on helophyte belts, such as *Carex* sp. and *Juncus* sp. Exuviae were collected in 2014 only on 4 ponds out of the 7 used for larval sampling (Table 2.1). They were sampled in the vegetation on pond banks and all preserved in pure ethanol.

In order to compare traits of teneral emerged from reared larvae with those of mature individuals, 51 flying adults (*i.e.* 34 males and 17 females) were caught on 4 ponds and their surroundings between 17 June and 29 August 2017 (Fig. 2.1; Table 2.1).

## 2.2. Larvae rearing

As feeding can be problematic, last instar larvae were first placed into small wire cages on the bank of a single pond (pond 9; see Table 2.1). A grid width of 5 mm allowed small invertebrates to enter and serve as preys but prevented *A. imperator* larvae from escaping, since the minimal head width of last instar was larger than 8 mm. When they reached the fifth stage of metamorphosis or later, larvae were then reared in outdoor cages at the lab. At this stage, the larval prementum starts to retract and larvae stop feeding (Corbet, 1957).

After sex determination, larvae were placed in individual 1.8 liter polypropylene boxes (La Bovida, CARTY) and reared until emergence. Each box was filled with water to about 7 cm height. Two 45 cm bamboo sticks were wrapped in a small string to improve adherence of larval claws and placed in each box to provide emergence supports. Wire locker were built out of 5 mm metallic grid to keep adults separated after emergence. One male and one female could be placed together into each locker. Just after emergence, adults were sexed and associated with their exuviae. Among the 104 individuals initially sampled, 87 were successfully reared until emergence and 78 could be measured and weighted for further statistical analyses.

### 2.3. Trait measurements of larvae and teneralis

Larval instars were classified following Benke (1970) and Ferreras-Romero & Corbet (1999) using head width and wing pad length, and categorized as follows: Fo (final instar), F-1 (penultimate instar), F-2 and F-3. In order to have a very accurate monitoring of the larval development during the days prior to ecdysis, Fo larvae were categorized among 6 stages of metamorphosis (M<sub>1</sub> to M<sub>6</sub>) according to Corbet (1957). These stages were determined based on changes in eyes shape, wing buds and labium (Appendix A<sub>1</sub> & A<sub>2</sub>). Sex was determined by the presence or absence of gonapophyses on the 9<sup>th</sup> abdominal segment. This structure is apparent on females at larval, exuvial and adult stages.

Total length, head width and wing pad length were measured on all larvae and exuviae preserved in ethanol using a GENERAL Metric Dial Calliper under stereomicroscope Leica MZ6. When a specimen body was bent, total length measurement was not considered in analyses. In the case of reared larvae, they were placed on a plastified graph paper and photographed. Total length was then read on the picture with a precision of 0.5 mm. Just after their emergence, reared teneralis were measured with the calliper to the nearest 0.1 mm and weighted to the nearest 0.01 g in a tared box using a DENVER MXX-612 scale. Measurements were also taken on their exuviae before their storage in absolute ethanol.

### 2.4. Mature adult trait measurements

Mature adults were put into a paper envelope and weighted in the field using an OHAUS Traveler TA152 scale. Total length and right hind wing length were also measured with the calliper. More details on measurements of larvae and adults are available in Appendix A<sub>3</sub>.

### 2.5. Data analyses

Means are given  $\pm$  standard deviation (SD). All statistical analyses were performed using R 3.4.1 software (R Development Core Team, 2017).

To identify larval instars, head width was plotted against wing pad length. Each point cloud was then graphically discriminated. As M<sub>5</sub> in *A. imperator* lasts only one or two days and was represented by only few specimens in the sample, stages of metamorphosis 5 and 6 were pooled.

Some previous studies report an effect of ethanol preservation on body mass of aquatic macroinvertebrates (Leuven et al., 1985; Méthot et al., 2012). However, only little is known about changes in length caused by this mode of preservation on benthic macroinvertebrates (*e.g.* no constant shrinking of specimens in Ephemeroptera larvae for ethanol concentrations ranging from 70% to 95% (von Schiller & Solimini, 2005; Heise et al., 1988)). An effect of the mode of preservation was already reported on the dragonfly *A. junius*, but without statistical assessment (Calvert, 1943). Nevertheless, using data of specimens preserved in different conditions can introduce a real bias in the results.

In this study on *A. imperator*, two groups of larvae were used. One group was composed of alive larvae and another group of dead larvae preserved in pure ethanol. Effects of ethanol preservation, metamorphosis stage and their interaction on total length of last instar larvae were tested. Since assumptions of normality and homoscedasticity of data were not verified, the non-parametric two-way analysis of variance Scheirer-Ray-Hare test was performed using the 'rcompanion' R package (Mangiafico, 2017). Length of Fo larvae preserved in ethanol ( $45.1 \pm 3.3$  mm) was not significantly different from live larvae ( $46.3 \pm 1.9$  mm; Scheirer-Ray-Hare test:  $H = 2.03$ ,  $P = 0.15$ ,  $df = 1$ ) and this, whatever the stage of metamorphosis (Scheirer-Ray-Hare test: interaction  $H = 3.11$ ,  $P = 0.53$ ,  $df = 4$ ). For further analyses, all larvae were therefore included regardless of the preservation conditions.

A Scheirer-Ray-Hare test was performed to examine effects of sex, instar and their interaction on total length of both larvae and exuviae. Exuviae of reared individuals were excluded from this analysis to ensure independency of the data between the different instars. A comparison between mean lengths of reared final instar larvae at the capture and mean lengths of their corresponding exuviae after emergence was also done using a paired Student's t-test after verifying normality of data.

Analyses on body mass and length of teneralis were first performed using mixed GLMM models in order to consider a potential random effect on ponds. However, the estimated variance explained by the random effect was particularly weak (*i.e.*  $1.10^{-8}$ ), suggesting that the intercept of the model for the population (*i.e.* fixed part) do not change when considering each pond. Therefore, a standard multiple regression was performed to investigate the effect of total length of larvae, total length of exuviae, sex, date of emergence, sampling pond and rearing duration on body mass of teneralis. Final model was selected based on Akaike Information Criterion (AIC) using a backward

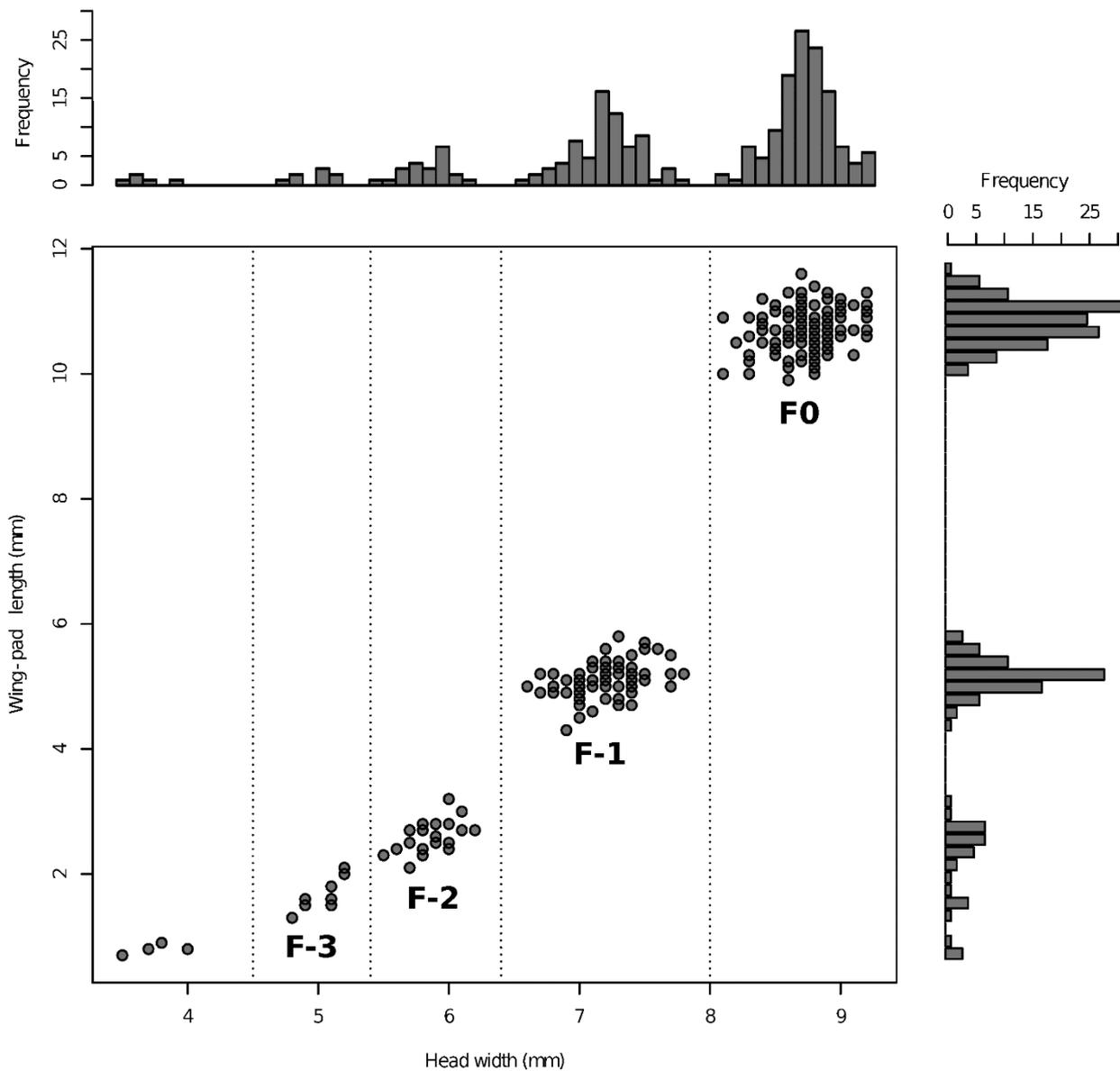
model selection. A generalized least squares (GLS) regression was used to investigate the effect of total length of larvae, total length of exuviae, sex, sampling pond, date of emergence and rearing duration on length of teneral. This analysis was used in order to correct the observed residual variance heterogeneity between sexes (Zuur et al., 2009; Zuur et al., 2015) and was performed using the 'nlme' R package (Pinheiro et al., 2018). A pseudo adjusted  $R^2$  was assessed using the 'rcompanion' R package (Mangiafico, 2017). Normality and homoscedasticity of residuals were systematically tested after regressions.

Effects of sex, age (*i.e.* teneral or mature) and their interaction were first tested on the total length of adults and then on their hind wing length, using a two-way analysis of variance (ANOVA), after verifying normality and homoscedasticity of the data. A Scheirer-Ray-Hare test was performed to investigate effects of sex, age and their interaction on the total body mass of adults. When necessary, post-hoc tests were performed after ANOVAs and Scheirer-Ray-Hare tests. Polynomial regressions were used to investigate the effect of the date of capture on the total length and on the body mass of mature individuals for each sex. Models were selected based on AIC using a backward selection. Finally, adult hind wing lengths were compared with values from the database of morphological and geographical traits of the British odonates (Powney et al., 2014) using Student's t-tests.

## 3. Results

### 3.1. Larval stages

Using a plot with measurements of wing pad length against measurements of head width, 5 point clouds related to the larval instars were graphically identified (Fig. 2.2). Frequency histogram of head width presented distinct modes for the 4 last instars. Frequency histogram of wing pad length showed very clear modes for F<sub>0</sub> and F<sub>-1</sub>, but did not enable to discriminate smaller instars. Threshold values for head width and wing pad length were thereby graphically defined for *A. imperator* in Normandy (Table 2.2).



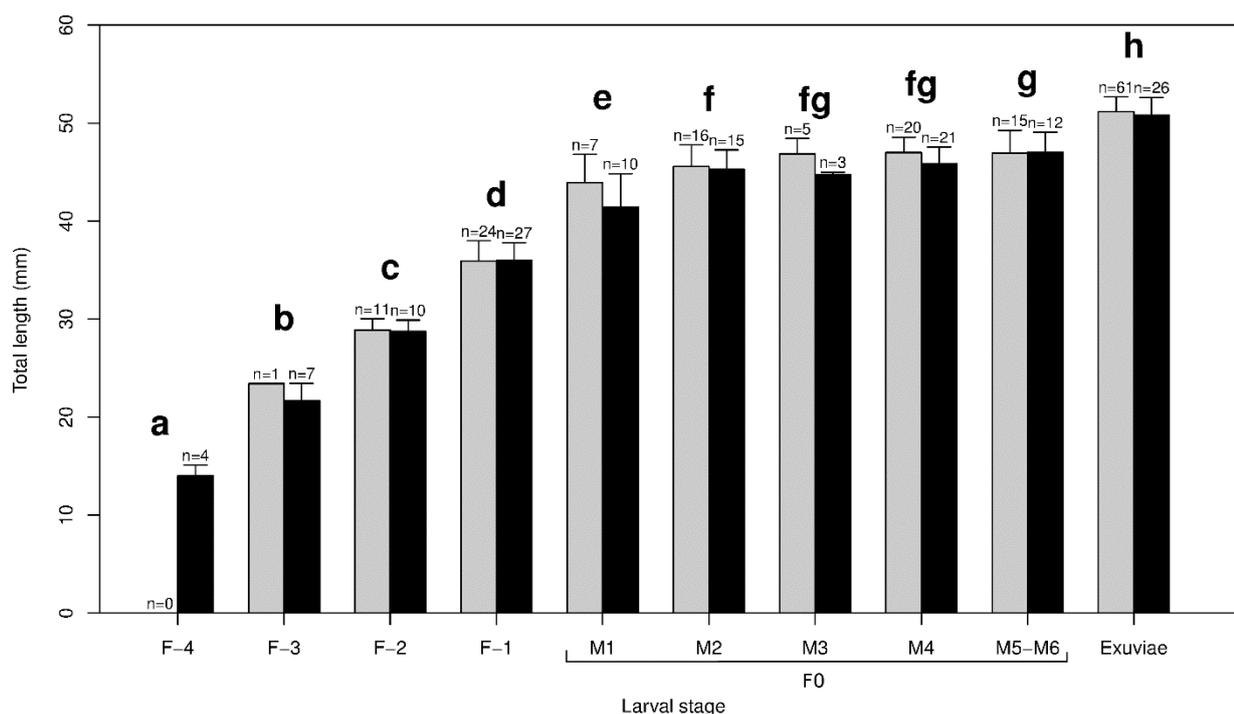
**Fig. 2.2.** Plot of the larval wing pad length against the head width of *A. imperator* larvae ( $n = 241$ ). Histograms show the frequency of measurements. Peak and valleys were used to identify larval instars. Extrapolated threshold values for head width are represented by dotted vertical lines.

**Table 2.2.** Thresholds identified graphically for head-width and wing pad length with the dataset of this study and used to separate larval instars. Values are given in mm.

	F-3	F-2	F-1	F <sub>0</sub>
Head-width	4.5 - 5.4	5.4 - 6.4	6.4 - 8	8 - 9.5
Wing pad length	NA	NA	4 - 6	9.5 - 12

There was a significant difference in total length between each instar from F-4 to exuviae and even within the different stages of metamorphosis of F<sub>0</sub> larvae (Scheirer-Ray-Hare test:  $H = 240$ ,  $P < 0.001$ ,  $df = 9$ ; Fig. 2.3). Females ( $45.3 \pm 7.2$  mm) were found to be significantly longer than males ( $41.1 \pm 9.4$  mm; Scheirer-Ray-Hare test:  $H = 19.7$ ,  $P < 0.001$ ,  $df = 1$ ) and no significant effect of the interaction between the sex and the stage of metamorphosis was found (Scheirer-Ray-Hare test:  $H = 0.884$ ,  $P = 0.999$ ,  $df = 8$ ).

In order to compare the length of reared larvae and their exuviae, only measurements done on larvae between M<sub>3</sub> and M<sub>6</sub> were pooled. Stages of metamorphosis M<sub>1</sub> and M<sub>2</sub> could not be included in this analysis since they were significantly different in length from stages M<sub>3</sub> to M<sub>6</sub> (Fig. 2.3). Exuviae ( $51.1 \pm 2.4$  mm) were significantly longer than their corresponding last instar larvae ( $45.9 \pm 1.8$  mm; Paired Student's t-test:  $t = 8.68$ ,  $P < 0.001$ ,  $df = 25$ ).



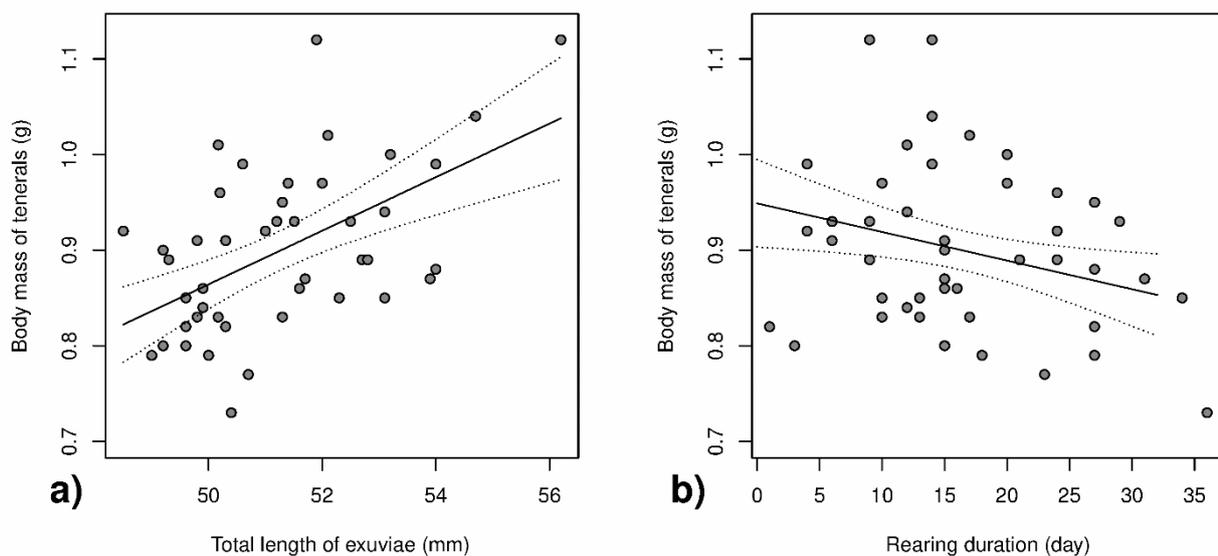
**Fig. 2.3.** Total length of females (in grey) and males (in black) larvae from collection (F-4 to exuviae) pooled with reared larvae (M<sub>1</sub> to M<sub>6</sub>). All measurements were taken from different individuals and exuviae of reared larvae were not included. Letters show significant differences in post-hoc analyses ( $\alpha = 0.05$ ). Values plotted are means + SD.

### 3.2. Adult traits

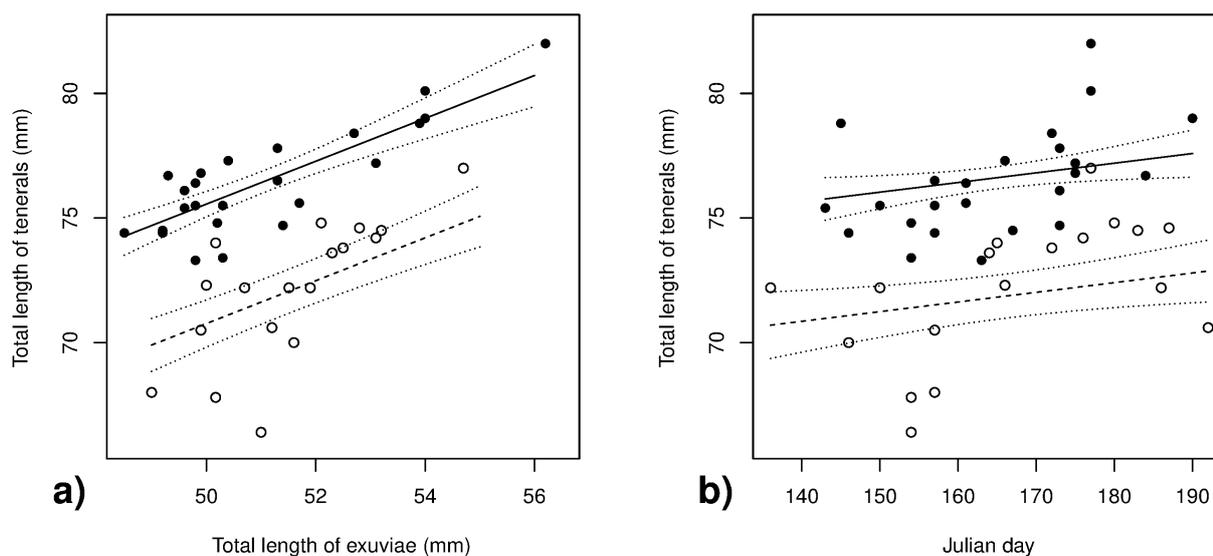
Body mass of teneral was significantly related to the total length of exuviae and rearing duration (Table 2.3, Fig. 2.4a & b). Total length of teneral was significantly related to total length of exuviae, sex and date of emergence (Table 2.3, Fig. 2.5a & b).

**Table 2.3.** Results of the regression analyses on predictors of body mass and total length of *Anax imperator* teneralis

Model summary	Selected variables	Estimate $\pm$ SE	Estimate p.value
<b>Body mass of teneralis</b>			
df = 42	Total length of exuviae	0.028 $\pm$ 0.006	< 0.001
Adjusted R <sup>2</sup> = 0.336	Rearing duration	-0.003 $\pm$ 0.001	0.021
<b>Total length of teneralis</b>			
df = 43	Total length of exuviae	0.860 $\pm$ 0.122	< 0.001
Pseudo adjusted R <sup>2</sup> = 0.792	Sex	4.794 $\pm$ 0.507	< 0.001
	Date of emergence	-0.039 $\pm$ 0.017	0.028



**Fig. 2.4.** Linear relationships of body mass of teneralis against total length of exuviae (a) and against rearing duration (b) of *A. imperator*. Dotted lines represent 95% confidence intervals (see Table 2.3 for details).

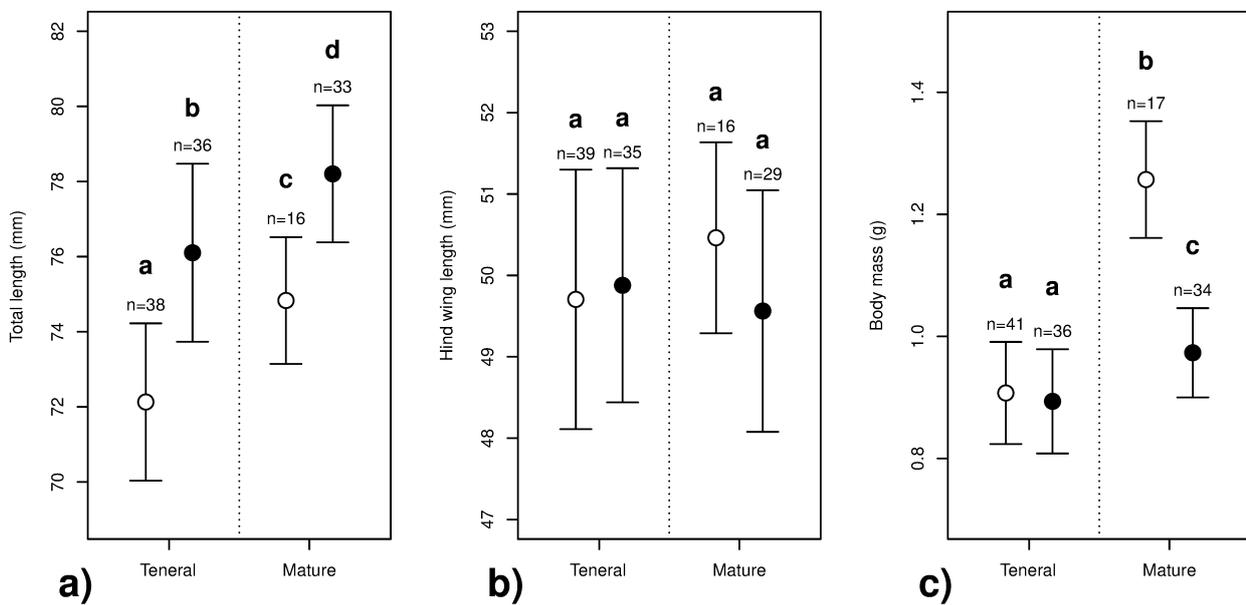


**Fig. 2.5.** Linear relationships of total length of teneral against total length of exuviae of *A. imperator* (a) and against the emergence date in Julian days (b). Opened dots represent females ( $n = 18$ ) and filled dots represent males ( $n = 25$ ). Dotted lines represent 95% confidence intervals (see Table 2.3 for details).

Mature individuals ( $77.1 \pm 2.4$  mm) were significantly longer than teneral (74.0  $\pm$  3.0 mm) and males ( $77.2 \pm 2.3$  mm) were significantly longer than females ( $72.9 \pm 2.3$  mm) at any age (Table 2.4, Fig. 2.6a). There was no significant difference in hind wing length according to sex, age, nor an interaction between both these factors (Table 2.4; Fig. 2.6b). The comparison of our data ( $49.8 \pm 1.5$  mm) with those of Great Britain ( $50.2 \pm 1.1$  mm) also showed no significant difference ( $t = 1.35$ ,  $P = 0.191$ ,  $df = 22.57$ ) in right hind wing length. There was an effect of sex, age and their interaction on body mass (Table 2.4; Fig. 2.6c). Mature individuals were significantly heavier ( $1.06 \pm 0.16$  g) than teneral ( $0.90 \pm 0.08$ ; Table 2.4; Fig. 2.6c). While no significant difference in body mass was found between teneral males and teneral females (Table 2.4; Fig. 2.6c), mature females ( $1.26 \pm 0.09$  g) were significantly heavier than mature males ( $0.97 \pm 0.07$  g; Table 2.4; Fig. 2.6c).

**Table 2.4.** Results of two-way analyses testing the effect of age, sex and their interaction on three traits of adult *Anax imperator*.

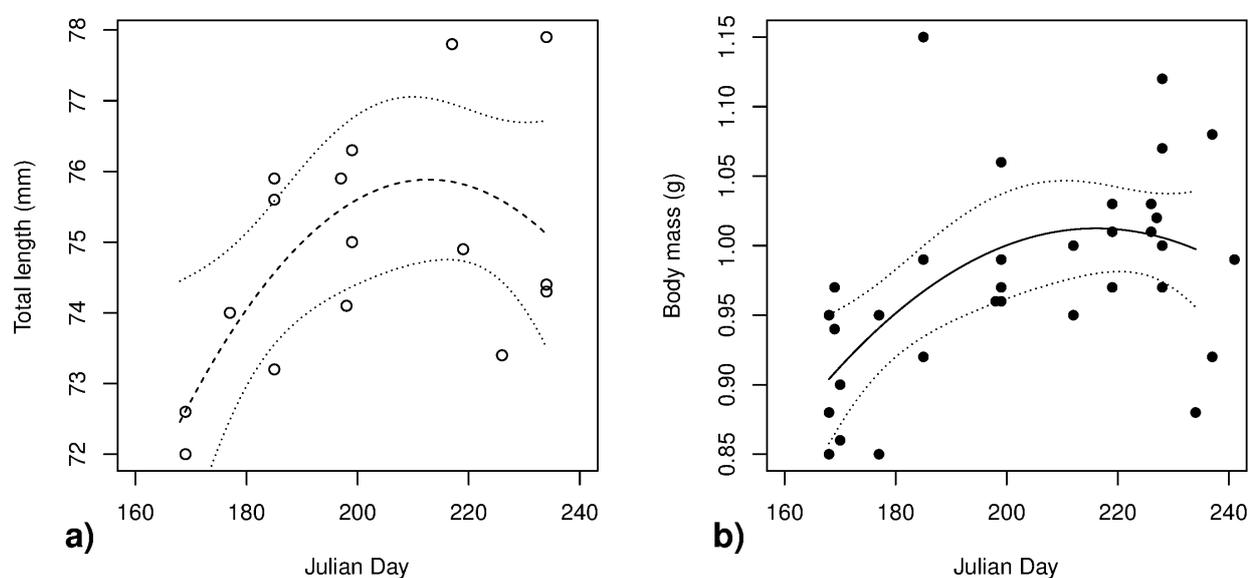
Variable	Method	Age	Sex	Interaction
Total length	Two-way ANOVA	Matures > Teneral ( $F = 36.5, P < 0.001$ )	Males > Females ( $F = 124, P < 0.001$ )	No ( $F = 0.578, P = 0.449$ )
Hind wing length	Two-way ANOVA	No difference ( $F = 0.248, P = 0.619$ )	No difference ( $F = 0.520, P = 0.472$ )	No ( $F = 3.45, P = 0.066$ )
Body mass	Scheirer-Ray-Hare	Matures > Teneral ( $H = 45.6, P < 0.001$ )	No difference ( $H = 3.01, P = 0.083$ )	Yes ( $H = 7.94, P = 0.005$ )

**Fig. 2.6.** Comparison of traits between reared teneral and mature adults caught in the field for each sex. Females are represented by opened dots and males by filled dots. Values plotted are the means  $\pm$  SD: total body length (a), total length of right hind wing (b) and total body mass (c). Letters show significant differences in post-hoc analyses ( $\alpha = 0.05$ ).

In mature individuals, body mass of males was significantly related to the sampling date (Table 2.5) and a theoretical optimum was found on Julian Day 216 (04/08/2017; Fig. 2.7a). Total length of females was also significantly related to the sampling date (Table 2.5) and a theoretical optimum was found on Julian Day 213 (01/08/2017; Fig. 2.7b). No significant relationship was found between the sampling date and female body mass or male total length.

**Table 2.5.** Results of the models testing for the relationship between total length or body mass against the sampling date in Julian days of *A. imperator*. Regressions were done on both sexes of mature individuals. Variables were selected using AIC. Regression coefficient ( $R^2$ ) is given for the final model using adjustment when more than one variable was selected. Model p.value below the 0.05 threshold are given in bold.

Age and sex	Response	Selected variables	Estimate $\pm$ SE	Estimate p.value	Model p.value	Model $R^2$
Mature males	Body mass	Julian Day	$0.020 \pm 0.010$	0.051	<b>0.004</b>	0.260
		Julian Day <sup>2</sup>	$-0.000 \pm 0.000$	0.068		
Mature females	Body mass	none	-	-	-	-
Mature males	Total length	Julian Day	$0.558 \pm 0.283$	0.058	0.104	0.085
		Julian Day <sup>2</sup>	$-0.001 \pm 0.001$	0.054		
Mature females	Total length	Julian Day	$0.156 \pm 0.065$	0.061	<b>0.046</b>	0.282
		Julian Day <sup>2</sup>	$-0.002 \pm 0.001$	0.072		



**Fig. 2.7.** Regressions between total length of mature females (a) and body mass of mature males (b) of *Anax imperator* against the sampling date in Julian days. Dotted lines represent 95% confidence intervals.

## 4. Discussion

Carry-over effects can be defined as changes in measurable traits induced by environmental conditions that persist over the seasons or subsequent stages of life (Pechenik, 2012; Harrison et al. 2011; Stoks & Córdoba-Aguilar, 2012). In Odonata, hatching date and larval conditions have a significant impact on adult traits, at least for damselfly species (Arambourou, 2017; Stoks & Córdoba-Aguilar, 2012, Tüzün & Stoks, 2018). However, more information is necessary to decipher mechanisms that are responsible for carry-over effects between larval and adult stages, especially in larger odonates. Based on a set of morphological measurements, this study contributes to bring new insights on the biometry of the large dragonfly *Anax imperator*.

A strong relationship was found between total length of exuviae and teneral, meaning that larval conditions would have an impact on the size of the new imago. In odonates, maturation corresponds to the period between emergence and reproduction during which individuals feed and acquire sexual maturity (Tyagi, 2007). After maturation, adults caught near the ponds were significantly longer and heavier than reared teneral. This significant growth in length during the maturation might be possible by the expansion of the endocuticle few days after emergence (Neville, 1983). Also, odonates might be able to compensate a low mass or size at emergence by a greater increase in body mass during their maturation (Hyeun-Ji & Johansson, 2015). During this period, body mass of studied females increased by an average of 39%, while only little gain of mass was observed for males. A previous study also found similar results in several dragonfly species, with even a loss of mass in males for some species. The greater increase in body mass of females is probably due to the development of ovaries associated with thoracic muscle to maintain flight performance (Anholt et al., 1991).

Females were significantly heavier than males, but rely on the same wing surface to fly. Nevertheless, this higher wingload is not necessarily a significant disadvantage for females, since conversely to males, they do not need to stay up in the air for a long time and to fight. For both sexes, differences in body size and mass between teneral and adults could be explained by a better survival rate of the larger individuals that generally benefit a better fitness and energy reserve to get over the maturation period (Marden & Rowan, 2000; Stoks & Córdoba-Aguilar, 2012). However, our data cannot fully support this hypothesis, since measurements of wing length do not show a similar increase. While rearing conditions had no impact on individual grow in length, a lower mass at emergence was found for larvae reared over a longer time. Hence, reared larvae might have had a

lower food availability than they would *in vivo*. Rearing might have induced the slight difference reported in body mass between reared teneral and caught adults, but not the body mass increase of 39% in females. Further investigation testing for differences in total length and body mass, before and after maturation on the same individuals, would be interesting to test these hypotheses and the potential effect of rearing.

Contrary to previous studies on odonates (review by Corbet, 1999; Falck & Johansson, 2000), teneral *A. imperator* showed a significant increase in body length and mass with the date of emergence for both sexes. This result could be explained by the fact that individuals emerging late in the season have more time to grow before emergence. In mature adults, an optimum in total length or mass was found near the end of the flying season. The increase in body length and mass at the beginning of the season can be related to the fact, that individuals emerging earlier with a lower body size and mass will probably result in smaller and lighter adults. The observed gain of mass in males could also be explained by a better survival rate of the heavier males (Waller & Svensson, 2017), which are therefore predominant at the end of the flying season. They need to fight and fly over a long time before mating (Jödicke, 1997), probably increasing the mortality of weaker individuals. After the observed optima, the prevalence of smaller and lighter individuals at the end of the flying period could be explained by harsher environmental conditions, such as lower food availability.

Sexual Size Dimorphism (SSD) in dragonflies can be either male- or female-biased, but there is a tendency of male-biased SSD in territorial species and female-biased SSD in non-territorial species (Serrano-Meneses et al., 2008). In our study, although larval females were longer than males, both sexes had the same body mass at emergence and adult males were significantly longer than females. This male-biased SSD of *A. imperator* is consistent with the territorial behaviour of this species, but not with Serrano-Meneses et al. (2007) results in northwestern Spain, where adult females of *A. imperator* were significantly longer ( $72 \pm 2$  mm) than males ( $69 \pm 1$  mm) at emergence. The difference in total length between both latitudes could be explained by Bergman's rule for males, since this study in northwestern France was conducted under higher latitudes and resulted in larger individuals. However, this rule is not sufficient to explain the similar size of females between both studies.

Intraspecific interferences between voltinism groups can impact the larval mortality of populations (Crowley et al., 1987). In dragonflies, voltinism greatly depends on temperature and

latitude (Corbet et al., 2006). In this study, measurements on the last larval instars seem to follow a unimodal distribution for each stadium and did not allow to distinguish among voltinism classes. Either the studied larvae were all univoltines, while they are rather expected to be at least partly semivoltine in Normandy (extrapolated from Corbet et al., 2006), or differences in voltinism did not affect head width in the three last larval instars.

Measurements of head width and wing pad length of larvae were very efficient to discriminate last larval instars and allowed comparisons with few data from other studies under different latitudes (Table 2.6). Thresholds and mean values measured for larval head width were smaller in our study than in those measured in England (Corbet, 1955) and growth rate between instars was higher. This difference in larval length and growth can be explained by the latitude and climate variation between Normandy and England. It is surprising that comparisons in the mean wing length of adults between the two regions showed no significant difference given that larval sizes were very different. It would be interesting to compare other traits than wing length, but unfortunately, no other dataset was available. Larval measurements from Switzerland were very similar to our values. Although situated slightly lower in latitude, sample sites in Switzerland were located in higher altitudes, probably resulting in similar mean temperatures as in Normandy. In the Azores, Lamelas-Lopez et al. (2017) found larger means for head width and lower growth rates, similar to those of Corbet (1955) in England. If not a misinterpretation from the authors of the paper, this result is quite unexpected and difficult to explain according to Bergmann's rule. In fact, larvae from lower latitudes should be much smaller and have a faster development than in studies from higher latitudes. These considerations highlight the lack of data on odonate traits all over the world and the need to better understand variations in larval development according to different latitudinal contexts.

**Table 2.6.** Values of larval head widths (in mm) reported from the published studies for the four last larval instars. Lines are ordered according to a latitudinal gradient

Reference	Country	Value	F0	F-1	F-2	F-3
Corbet (1955)	England (51°N)	1 larva	9.0	8.1	7.2	6.2
Minot <i>et al.</i>	France (49°N)	Mean ± SD	8.7 ± 0.2	7.2 ± 0.2	5.9 ± 0.2	5.0 ± 0.2
Portmann (1921)	Switzerland (47°N)	NA	9.0	7.3	6.0	NA
Robert (1958)	Switzerland (NA)	Min – Max	8.5 – 9.2	6.9 – 7.7	5.7 – 6.1	4.9 – 5.5
Lamelas-Lopez <i>et al.</i> (2017)	Spain (38°N)	Mean ± SD	9.6 ± 0.3	8.8 ± 0.3	7.9 ± NA	6.9 ± 0.3

In line with previous results on *Aeshna cyanea* Müller, 1764 (Goretti et al., 2001), significant difference was found between the total length of last instar larvae and their exuviae. More surprisingly, an increase in total length within the metamorphosis stages of the last instar was highlighted. Since it is not possible for sclerites to change size except during moulting, they probably overlap in early stages of metamorphosis. Then, their exoskeleton might stretch out, resulting in an increase of the total larval length. This result emphasizes the importance of taking stages of metamorphosis into account in studies considering larval traits of odonates.

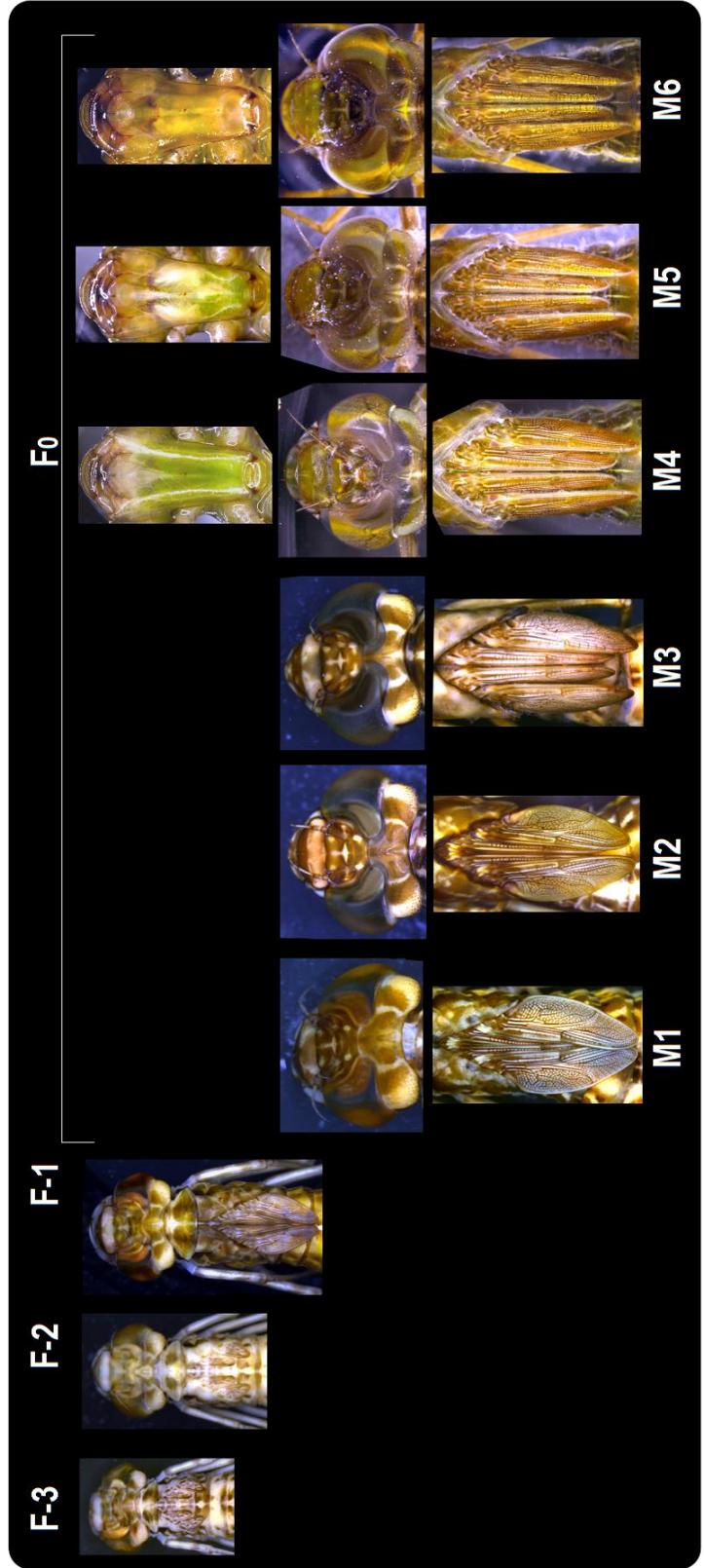
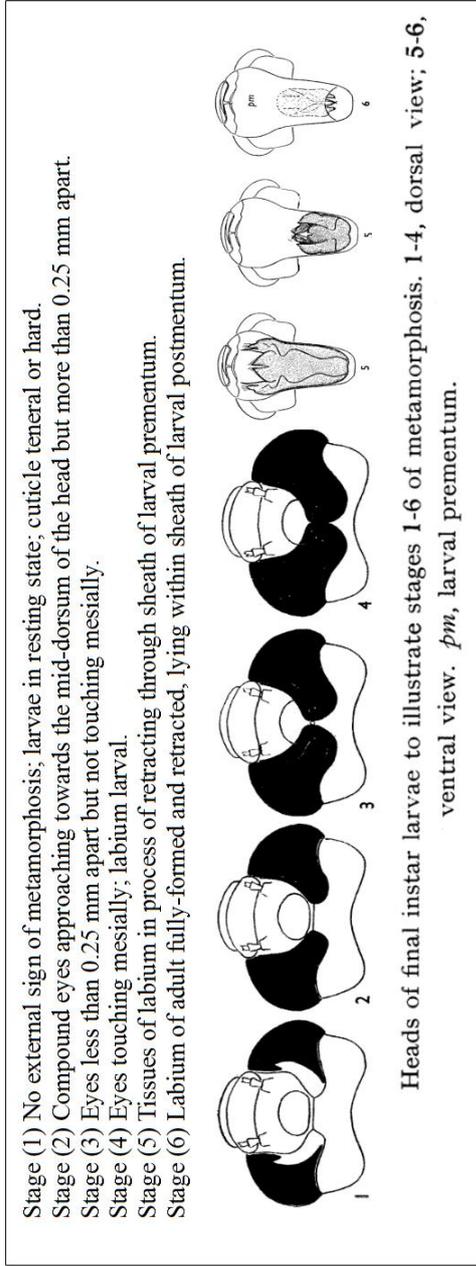
Overall, our results highlight the importance of taking into account the whole life cycle to understand fitness, behaviour and dispersal capacity of large dragonflies. In *A. imperator*, we found a relationship between larval and teneral traits, demonstrating a significant effect of early stage development on the morphology of adults. Further investigation is needed to fully understand the voltinism and mechanisms of larval development under different latitudes. Nevertheless, we can already state that there is a large heterogeneity depending on climatic conditions or environmental perturbations. Emerging theories connecting traits to ecological functions of individuals now enable to predict species abundance and community assembly (McGill, 2006; Laughlin et al., 2012), opening new perspectives for trait-based-ecology. Hence, trait studies are a crucial background to understand variations at local and larger scales and enhance further investigations on dispersal and population dynamics.

## 5. Acknowledgements

Thanks are due to Hippolyte Terrones for his help during the laboratory work and field collections. We also thank Lucie Mundell for English language reviewing.

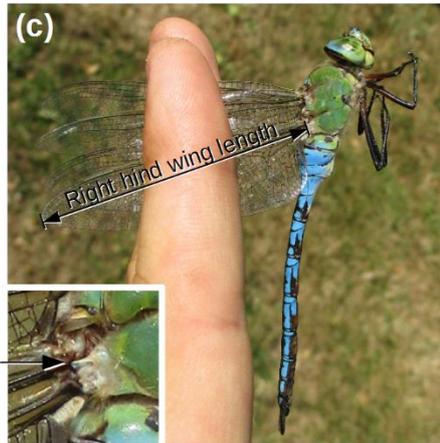
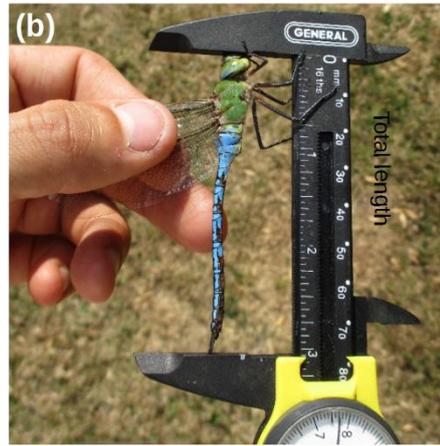
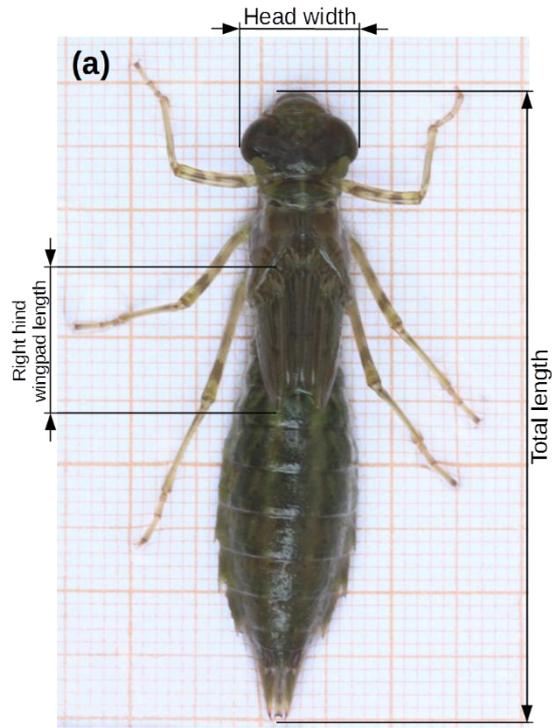
## 6. Supplementary materials

**Fig. 2.S1.**  
 Definition of the 6 stages  
 of Metamorphosis  
 (from Corbet 1957)



**Fig. 2.S2.** Illustrations of the morphological changes during larval growth. (pictures F-3 to M3 : larvae from collection; M4 to M6 reared larva)

Fig. 2.S3. Measurement method used for physical traits of larvae (a) and adults (b, c) *Anax imperator*





## **Chapter 3**

# **Effects of water pollution on the larval development and condition of the adults at emergence in *Aeshna cyanea* (Odonata: Aeshnidae)**

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### ***Preliminary results for future publication***

#### **Abstract**

In organisms with a complex life cycle, environmental perturbations during the larval development can have a negative carry-over effect on traits and fitness of the adults. At sublethal concentrations, water pollution can affect development duration, morphological traits, energy budget and cause a physiological stress in many species of Zygoptera. However, these effects were rarely investigated in larger species.

In this study, 179 larvae of *Aeshna cyanea* (Anisoptera) were reared in water containing different concentrations of Roundup or DEET (*i.e.* 1-3 µg.L<sup>-1</sup>, 100-300 µg.L<sup>-1</sup> and 10-30 mg.L<sup>-1</sup> and controls). At the end of the study, 91 individuals had emerged successfully, 75 were still at the larval stage and 13 had died. Morphological traits of larvae were measured across their successive moults, as well as those of adults at emergence. The level of Hsp70 stress protein in the head of all larvae et adults was also quantified at the end of the experiment using Western blots.

Our results show that the larval traits, especially the tibia length presented a strong correlation with adult traits. Adults traits were also strongly correlated with each other. The growth rate differed among the larval stages, but was not affected by the individual sex or treatment. Adult traits were strongly related to the sex of individuals, but did not present any difference according to the treatment of the larva. Finally, the level of Hsp70 was significantly higher in adults than in larvae, but individuals that were exposed to Roundup or DEET did not differ from those of the control treatment.

This study provides insights into the development of *A. cyanea* larvae and biometric relationships across successive stages. However, no effect of the pollutants could be detected on individual traits or physiological stress of both larvae and adults.

**Key words:** Water pollutants, Dragonfly, Heat-shock protein, Larval development, Traits

## 1. Introduction

Environmental conditions often affect growth rate and body size of the individuals leading to disparities between populations (Peacor 2007). In organisms with a complex life cycle, morphological traits of larvae such as body size or duration of the larval stages can be used as indicators to predict the future condition of the adults (Marshal & Keough 2005; Pechenik 2006). In insects or amphibians, any stress encountered during the early stages of development can have carry-over effects on the adult life (Van Allen et al. 2010; Debecker et al. 2017). However, these carry-over effects are often hard to assess because compensatory mechanisms might temporarily mask them (Stoks & Córdoba-Aguilar 2012). For instance, after a period of starvation, larvae can compensate for a low body mass by a higher food intake (Stoks et al. 2006). However, this process is often associated with an increase in oxidative stress that can have delayed consequences on the fitness of the adults after emergence (Janssens & Stoks 2020). The level physiological stress caused by environmental perturbations may also be evaluated by dosage of stress protein like the Heat Shock Proteins (*i.e.* Hsp60, Hsp70 and Hsp90) that are produced in insects (King & MacRae 2015). Especially, the Hsp70 may be used as a biomarker due to its high sensitivity to chemical substances (Moreira-de-Sousa et al. 2018).

Dragonflies are insects with an aquatic larval stage and a flying imaginal stage (Suhling et al. 2015). At temperate latitudes, the larval development often lasts much longer than the adult stage (Corbet 2006) and larvae also have a low mobility compared to the adults (Bilton 2001). They are therefore more susceptible than adults to be affected by environmental perturbations during their development and are in most cases unable to move away. Some larval traits like body size or activity levels (*e.g.* frequency of movements, foraging behaviour) were shown to affect directly adult traits (Mikolajewski 2007; Brodin 2009), but for others larval traits, especially physiological traits, the relationship is harder to establish (Stoks & Córdoba-Aguilar 2012). Environmental perturbations such time constraints (*e.g.* due to summer droughts) or food shortage during the larval development, led to a lower mass at emergence in *Lestes viridis* (De Block & Stoks 2005). The stress caused by the presence of predators also leads to a decrease in foraging activities at larval stage and results in a lower body mass at emergence in several species (Dmitriew & Rowe 2005; De Block et al. 2008). These low energy reserves at the beginning of the adult life may be compensated by higher foraging activities during the maturation period but it increases the risk of being caught by predators (Stoks 2001).

In anthropized areas, a large volume of biocides (*e.g.* for agricultural purposes or to prevent human diseases) is released each year in the environment and often ends up in freshwater systems (Costanzo et al. 2007; Moss 2008). Exposure to these chemical substances can impair the development of aquatic larvae and lead to the death of individuals at high concentrations (Finotello et al. 2017). At sublethal concentrations, larval exposure to pesticides can affect the adult traits leading to smaller adult size or wing asymmetry (Hardersen & Wratten 1998; Janssens & Stoks 2013; Stoks et al. 2015) but these effects can be hard to assess on the field (Hardersen et al. 1999). Pesticides can also delay the egg hatching date, leading to a strong carry-over effect on the lifetime mating success of adults (Tüzün & Stoks 2017). The response of larvae to water pollution often interacts with other factors like water temperature, and the combined effects may yield to an even higher effect on the energetic budget (*i.e.* reserve of protein, sugar and fat) or lethality (Dinh 2016; Verheyen & Stoks 2020). Although the effects of water pollution on adult condition have often been investigated in zygopteran, these carry-over effects have rarely been investigated in larger dragonfly species (Stoks & Córdoba-Aguilar 2012).

In the present study, we investigated the effect of an insect repellent (*i.e.* DEET) and an herbicide (*i.e.* Roundup) on the larval development and adult condition of 197 individuals of *Aeshna cyanea* reared at different concentrations. Morphological traits were measured on larvae after each moult as well as on adults just after emergence. The level of the Heat-shock protein 70 (Hsp70) was also quantified on adults just after emergence and on the remaining larvae at the end of the experiment using Western blots. These protein quantification assays enabled to quantify the stress in each individual and compare its level between life stages and different levels of exposure to pollutants.

## 2. Material and methods

The study was carried in the agglomeration of Rouen in the Normandy region, North-Western France. The climate of this region is oceanic temperate with a mean annual air temperature of 10.5°C and total annual precipitation averaging 851.7 mm (Météo France, 2020).

### 2.1. Study species and water pollutants

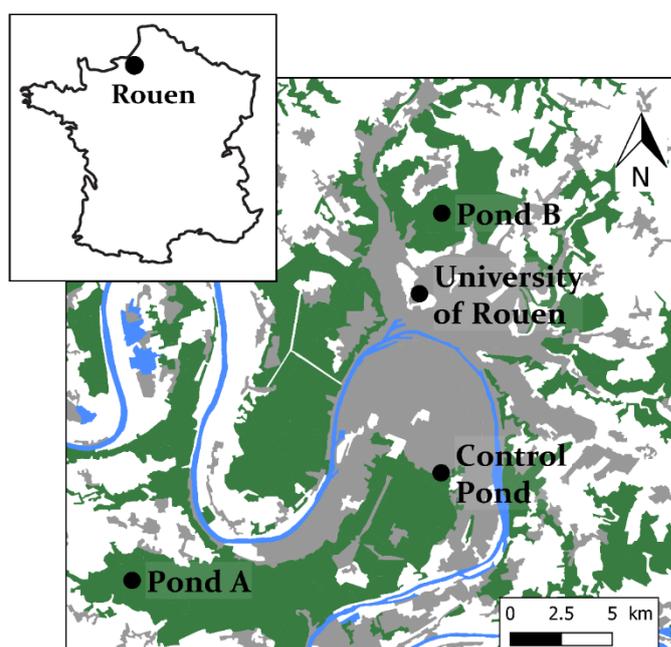
*Aeshna cyanea* is a species widely distributed in Europe and one the most common Aeshnidae of the Normandy region, France (Goretti et al. 2001; CERCION 2017). This large dragonfly measures between 67 and 76 mm and can be very abundant in forest or urban ponds (Dijkstra 2015; Márquez-Rodríguez 2020). The larvae are essentially univoltine or semivoltine at the study latitude (Corbet et al. 2006). They moult 10 to 13 times before ecdysis and the final instar can reach up to 50 mm in length (Goretti et al. 2001; Corbet 2002). This species was chosen for the study, especially because the larvae can be found in high densities on the forest ponds of the study region. Moreover, the rearing conditions of temperature and light at the laboratory were closer to those in forest ponds conditions than to warm sun-exposed ponds.

The Roundup® is an herbicide used in worldwide crop cultures and contains essentially glyphosate, diquat dibromide (DD) and a surfactant which is often polyethoxylated tallow amine (POEA; Daouk et al. 2013; Reddy et al. 2018). This chemical mixture can be lethal (96h-LC<sub>50</sub>) to amphibians species at concentrations ranging from 0.8 to 3.2 mg.L<sup>-1</sup> (Relyea & Jones 2009) and for *Daphnia* sp. at 25.5 mg.L<sup>-1</sup> (Tu et al. 2001). Low concentrations of Roundup also affect the growth of some aquatic macro-invertebrates like *Chironomus* sp. or feeding activities of *Gammarus* sp. (Ferreira-Junior et al. 2017; von Fumetti & Blaurock 2018). The DEET (N,N-Diethyl-meta-toluamide) is the most common insect repellent often used against mosquitoes or other biting insects (Ditzen et al. 2008). Lethal concentrations (48h-LC<sub>50</sub>) have been found at 80 mg.L<sup>-1</sup> for the caddisfly *Sericostoma vittatum* (Campos et al. 2016) and at 160 mg.L<sup>-1</sup> for *Daphnia magna* (Seo et al. 2005). Nevertheless, no information on the impact of these water pollutants on odonates was available.

## 2.2. Larvae rearing

Eight larvae of *Aeschna cyanea* were collected the 13 June 2018 on the pond A and 189 were collected the 19 June 2018 on the pond B (Fig. 3.1).

Among the 197 collected larvae, 15 final instar larvae (*i.e.* 8 from pond A and 7 from pond B) were reared in a Control Pond (CP) with similar conditions to the sampling ponds between 22 June and 7 August 2018 (Fig. 3.1). These individuals reared in situ were used to compare the level of stress with individuals reared at the laboratory. They were placed into 30 × 30 × 10 cm wire cages with 5 mm mesh width. These cages allowed small preys to enter but prevented the larvae from escaping. Their use was only suitable for the last instar larvae (Fo), since smaller instars could pass through the mesh. The cages were installed on the bank of the pond CP, half submerged in water. Over the entire rearing period, mean air temperature was  $21.2 \pm 5.2^{\circ}\text{C}$  and mean sun radiation during daytime was  $127 \pm 155 \mu\text{mol}/\text{m}^2\text{s}$ .



**Fig. 3.1.** Localisation of the sampling ponds A and B, the Control Pond (CP), and the laboratory of the university. Forest are represented by green polygons, urban areas by grey polygons and water surfaces by blue polygons.

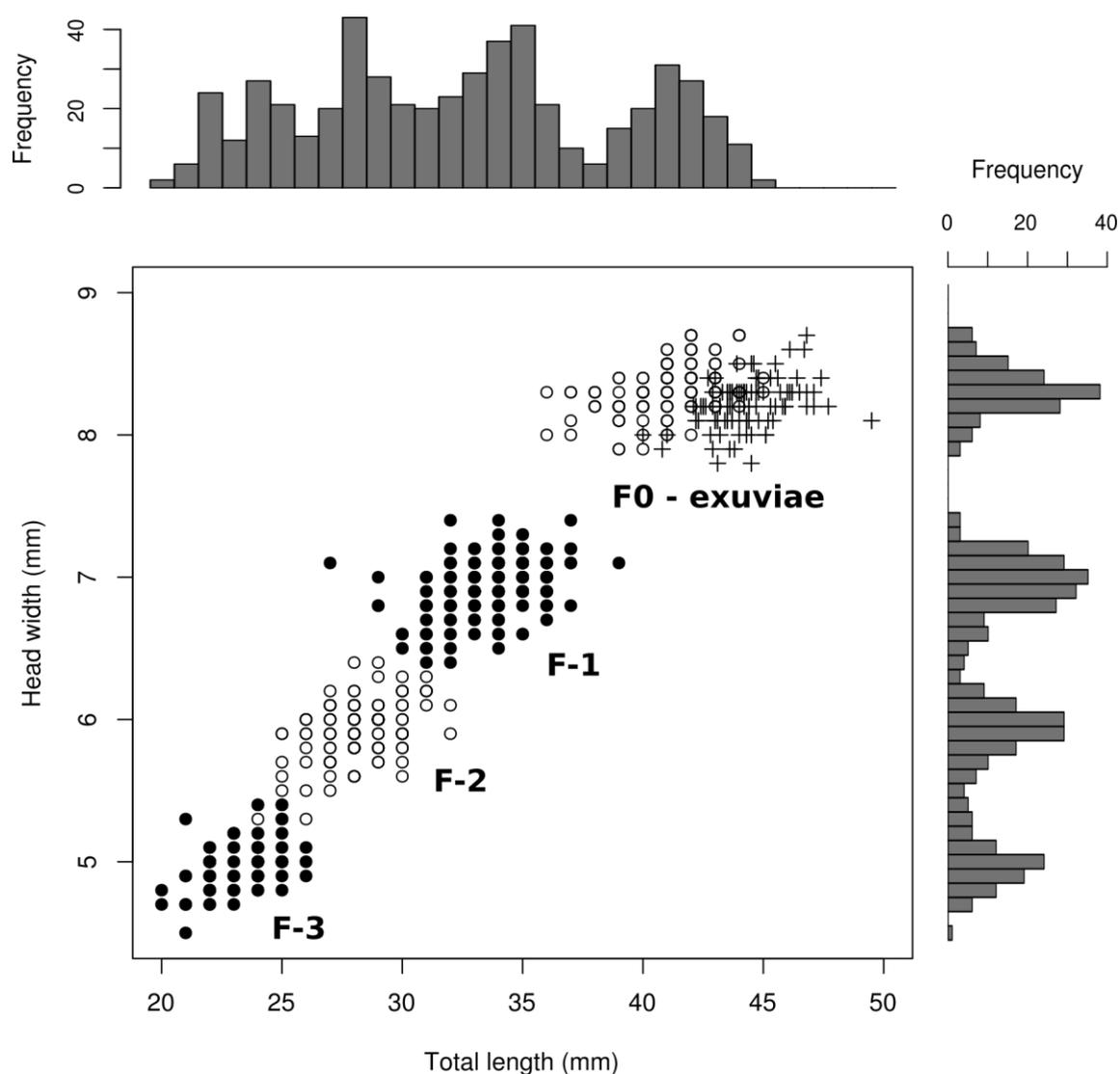
The other 182 larvae were reared at the laboratory between 27 June and 5 October 2018. Each larva was placed in an individual polypropylene box (La Bovida 1.8 L, CARTY) to prevent cannibalism which is quite common in this species at high larval densities (Moens 1973; Buskirk 1992). Each box was filled with water to about 2 cm and one leave of beech tree collected in the sampling pond was added to provide a natural substrate and a shelter. Over the entire rearing period, the mean air temperature of the room was  $17.2 \pm 0.91^{\circ}\text{C}$  (Hobo Pro v2, Onset) and mean sun radiation was  $7.6 \pm 14.3 \mu\text{mol}/\text{m}^2\text{s}$  during daytime (PAR Photon Flux Sensor, Model QSO-S). The larvae were fed ad libitum with commercial *Chironomus sp.* six days per week.

### 2.3. Trait measurements and instar identification

First measurements were taken on the 22 June 2018 on larvae reared in the control pond (CP) and on the 27 June 2018 on all larvae reared at the laboratory. Each larva was put on a plasticised graph paper to measure its total length. Head width and length of the metatibia were also measured using a GENERAL Metric Dial Calliper. These measurements were then repeated after each moult.

During the last instar, when larvae entered in the fifth stage of metamorphosis, characterised by mentum retraction (Corbet, 1957), the boxes were placed into small wire cages of 5 mm metallic mesh. Emergence support was provided by a 10 cm wide piece of nylon net tied to the edges of the cage. After emergence, adults were left in the cage a few hours until the cuticle and wings hardened. The total length, head width and length of the metatibia of exuviae were measured using the calliper. Adults were then carefully taken to measure their total length, head width, and right hind wing length with the calliper. Their body mass was also recorded to the nearest 0.01 g using a DENVER MXX-612 scale.

Larval instars were categorized following Goretti et al. (2001) from the last instar denoted Fo to smaller instars denoted F-1 (*i.e.* penultimate), F-2 and F-3. For all individuals that had emerged by the end of the experiment ( $n = 107$ ), the larval instars were *a posteriori* attributed by counting the number of moults before ecdysis. For individuals that did not emerge during the experiment ( $n = 75$ ), the instars were attributed by comparing the measurements of head width to the ones of the individuals that had emerged (Fig. 3.2).



**Fig. 3.2.** Plot of larval head width against total length for the last larval instars of *A. cyanea* larvae. Histograms show the frequency of measurements. Larvae assigned to the instars F-3 and F-1 are represented by filled circles, instars F-2 and F0 are represented by open circles and exuviae are presented by 'plus'. A total of 357 points was used build this graph, based on measurements of the 197 reared larvae.

#### 2.4. Experimental setup

At the laboratory, from the 27 June 2018, 26 larvae were assigned to 7 different treatments containing either Roundup (R), DEET (D), or only tap water for a Laboratory Control (LC; Table 3.1). Sexes and different larval instars were equitably distributed among the treatment groups. These treatments were denoted as follow: D1, D2, D3, R1, R2 and R3 corresponding respectively to concentrations of 10 mg/L, 100 µg/L and 1 µg/L of DEET and Roundup (Table 3.1). They were prepared in seven 200 L plastic tanks filled with tap water and left during several days for

dechlorination. Treatments D<sub>1</sub> and R<sub>1</sub> were prepared by adding 2 g of DEET and Roundup in the tanks and mixing with a bamboo stick. Two litres were then taken from these tanks and used to prepare D<sub>2</sub> and R<sub>2</sub>. Similarly, two litres of these treatments were used to prepare D<sub>3</sub> and R<sub>3</sub>. To prepare the control, two litres of tap water were added in a seventh tank LC. Since the pollutants were expected to degrade after several days in the tanks, new pulses respecting the same theoretical concentrations (*i.e.* 10 mg/L, 100 µg/L and 1 µg/L) were added fifteen days after the beginning of the experiment (*i.e.* the 11/07/2018) and one month after (*i.e.* the 27/07/2018). All individual boxes were washed twice a week and their water was replaced by water coming from the appropriated tanks. After emergence, the head of each individual was placed in RNA later and preserved at - 80°C for further analyses. The head of larvae that had not emerged at the end of the experiment were also collected on the 05 October 2020.

**Table 3.1.** Detail on the number of females and males used in each treatment for each larval instar.

Treatment	Details	Number of Females / Males used				
		F-3	F-2	F-1	Fo	Total
D <sub>1</sub>	DEET 10-30 mg.L <sup>-1</sup>	8/5	5/2	5/1	0/0	18/8 (26)
D <sub>2</sub>	DEET 100-300 µg.L <sup>-1</sup>	8/6	4/1	5/2	0/0	17/9 (26)
D <sub>3</sub>	DEET 1-3 µg.L <sup>-1</sup>	8/4	3/3	6/2	0/0	17/9 (26)
R <sub>1</sub>	Roundup 10-30 mg.L <sup>-1</sup>	6/4	8/2	4/2	0/0	18/8 (26)
R <sub>2</sub>	Roundup 100-300 µg.L <sup>-1</sup>	8/4	5/2	5/2	0/0	18/8 (26)
R <sub>3</sub>	Roundup 1-3 µg.L <sup>-1</sup>	7/4	6/2	6/1	0/0	19/7 (26)
LC	Laboratory control	7/4	6/3	6/0	0/0	19/7 (26)
CP	Control pond	0/0	0/0	0/0	11/4	11/4 (15)

## 2.5. Stress protein assay

Western blot assays were used to quantify the level of the stress protein Hsp70. The heads were first homogenized in 500 µL of Tris-Buffer (0.1 M, pH 7.5 and a protease inhibitor) using a FastPrep (6.5 m.s<sup>-1</sup>, 40 seconds; MP Biomedicals) and centrifuged for 3'30 min (16,696 × g , 4°C). The supernatant was then collected in an Eppendorf tube. This operation was repeated twice and final protein concentration in each sample was determined using a Pierce Bicinchoninic Acid (BCA) assay kit (ThermoFisher). A volume equivalent to 25 µg of protein was then diluted with ultrapure water and 6 µL of Loading Sample Buffer (5× concentrated), such that the final volume equals 30 µL. The mixture was boiled during 1 min and loaded on a Nu Page 4-12% Bis-Tris Protein Gel (ThermoFisher). To enable comparison between plots, a control sample containing 2 µg of HeLa cell (Enzo Life Science ADI-LYC-HL102) was also added on each gel.

After migration, proteins were transferred to a nitrocellulose membrane (Amersham Protan Premium 0.45 $\mu$ m). Hsp70 were detected using a first antibody (dilution 1:1500, Polyclonal Rabbit Anti-Human HSP70, SPC-103C Gentaur) and a conjugated secondary HRP antibody (dilution 1:30,000, Goat anti-Rabbit IgG, Invitrogen). Revelation was done by ECL (West Pico PLUS, Thermo Fischer) using a Vilber Fusion FX imager. The optic density of the Hsp70 protein images was then measured on the images using the software ImageJ (Schneider et al. 2012). The final level of protein was expressed in percentage of optic density compared to the control HeLa cell.

## 2.6. Data analysis

All statistical analyses were performed using R software (version 3.6.2; R Core Team, 2019). Means are given  $\pm$  SD and the model estimates are given  $\pm$  SE.

The proportion of mortality was compared between treatments using a Pearson's chi-squared test. To test for an effect of the larval development on the traits of adults after emergence, the relationships between traits of the larvae, exuviae and adults were tested using Pearson's correlation tests.

The growth rates of total length, head width, and tibia length between each larval instar were then investigated. These rates were calculated by dividing the value of measurement of a given instar by the same measurement done on the previous larval instar. General Linear Models (GLM) were used to test for an effect of the water pollution, sex and larval instar on these growth rates. The global significance of the factors used in the models was then checked by performing a Type II Anova with the R package "car" (Fox & Weisberg 2019). Effect of the water pollution and sex on the traits of adults after emergence was then tested using two-way ANOVAs. When condition of homoscedasticity was not met, a Sheirer-Ray-Hare test using the R package "rcompanion" (Mangiafico, 2017) was performed instead.

Finally, Generalized Linear Models (GzLM) were used to test for a relationship between the optical density of Hsp70 protein and the variables sex, stage at the end of the experiment (*i.e.* larva or adult) and treatment. These variables were tested for addition and interaction in the models with the R package "lme4" (Bates et al. 2015). Only the model with the lowest AIC after a backward selection was kept for interpretation of the results. In order to obtain a normal distribution of the residuals, an arcsin-square-root transformation of the percentage of optical density was applied

(Thomas 2017). To account for a potential inter-gel variability, the gel number was included as a random effect in the model. Individuals that were found dead were excluded for this analysis since the protein might have degraded before preservation at  $-80^{\circ}\text{C}$ . The global significance of the factors used in the models was checked by performing a Type II Anova with the R package “car” (Fox & Weisberg 2019). Calculation of the least square means for each category and post-hoc tests were then performed using the R package “emmeans” (Lenth 2019).

### 3. Results

In the control pond (CP), all 15 final instar larvae emerged successfully in the cages between 06 July and 07 August 2018. Larvae reared under laboratory conditions had not all emerged by the 05 October 2018. Among the 182 initial larvae, 91 individuals emerged successfully, 11 individuals died during the emergence, 4 died at the larval stage, 1 escaped and 75 were still at the larval stage at the end of the experiment. Death or emergence failure were respectively: 3 individuals in D1, 4 in D2, 1 in D3, 2 in R1, 1 in R2, 2 in R3, 2 in LC and 1 in CP. There was no significant difference in mortality according to the treatment (Chi-squared = 3.56, p-value = 0.827).

The correlation tests between traits showed correlations between the traits of larvae and the associated adult traits and this, from F-3. At all studied instars, the tibia length of the larva showed a significant positive correlation with the tibia length of the adult (Table 3.2). The head width of the last larval instar and of the exuviae was significantly positively correlated with the head width of the adult (Table 3.2). Correlations between the traits of exuviae and adults were significant only for the total body length (Table 3.2).

**Table 3.2.** Results of the correlation tests between the traits of larvae, exuviae and adults after emergence. The number of individuals (*n*) correspond to the number of larvae that were successfully reared to the emergence of the adult. Values below the  $\alpha = 0.05$  threshold are printed in bold.

Stages	n	Tibia length		Head width		Total length	
		r	P-val	r	P-val	r	P-val
Exuvia and adult	107	<b>0.75</b>	< <b>0.001</b>	<b>0.37</b>	< <b>0.001</b>	<b>0.28</b>	<b>0.006</b>
Fo and adult	107	<b>0.54</b>	< <b>0.001</b>	<b>0.35</b>	< <b>0.001</b>	0.14	0.139
F-1 and adult	94	<b>0.62</b>	< <b>0.001</b>	0.20	0.0568	0.04	0.712
F-2 and adult	48	<b>0.51</b>	< <b>0.001</b>	0.20	0.165	0.19	0.200
F-3 and adult	16	<b>0.50</b>	<b>0.048</b>	0.24	0.376	-0.19	0.496

Many traits measured on the adults presented correlation with each other. Only body mass and head, body mass and total length, and tibia length and wing length were not significantly correlated (Table 3.3). All other variables presented a significant positive relationship with each other (Table 3.3).

**Table 3.3.** Results of the correlation tests between the traits of adults ( $n = 107$ ). Values below the  $\alpha = 0.05$  threshold are printed in bold.

Adult trait	Total length		Body mass		Head width		Wing length	
	r	P-val	r	P-val	r	P-val	r	P-val
Tibia length	<b>0.28</b>	<b>0.004</b>	<b>0.26</b>	<b>0.016</b>	<b>0.21</b>	<b>0.030</b>	0.18	0.066
Wing length	<b>0.32</b>	<b>&lt; 0.001</b>	<b>0.25</b>	<b>0.019</b>	<b>0.56</b>	<b>&lt; 0.001</b>	-	-
Head width	<b>0.53</b>	<b>&lt; 0.001</b>	0.02	0.87	-	-	-	-
Body mass	0.19	0.083	-	-	-	-	-	-

The growth of larvae in total length, head width or tibia length between successive instars did not change according to the sex or the treatment in which individuals were reared (Table 3.4). However, the growth in total length was significantly higher between F-1 and Fo than between F-2 and F-1. The growth in head width was also significantly higher between F-1 and Fo, and between F-3 and F-2, than between F-1 and F-2. Finally, the growth in tibia length increases significantly with larval instars, since it was higher between F-1 and Fo than between F-2 and F-1 and higher between F-2 and F-1 than between F-3 and F-2 (Table 3.4).

**Table 3.4.** Results of the GLM of larval growth according to the sex, larval instar and treatment. Transition from larval instar F-3 to F-2 is denoted F<sub>32</sub>, transition from F-2 to F-1 is denoted F<sub>12</sub> and transition from F-1 to Fo is denoted F<sub>10</sub>. Values below the  $\alpha = 0.05$  threshold are printed in bold.

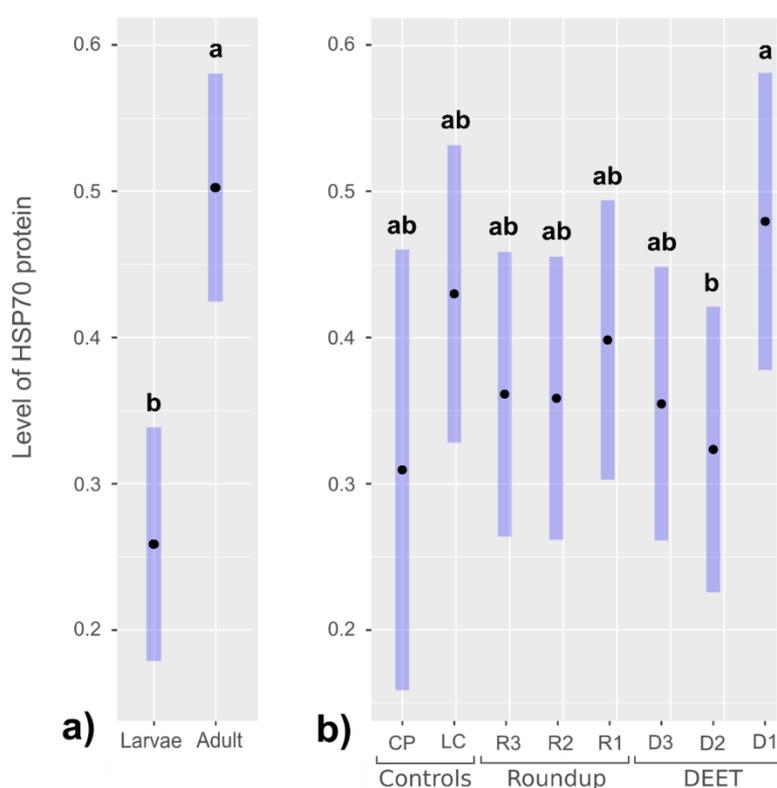
Response variable	Factor	Statistic	P-val	Post-hoc test
Growth in total length	Sex	0.280	0.596	-
	<b>Larval instar</b>	<b>3.421</b>	<b>0.034</b>	<b>F<sub>21</sub> &lt; F<sub>10</sub></b>
	Treatment	0.517	0.796	-
Growth in head width	Sex	0.925	0.337	-
	<b>Larval instar</b>	<b>9.532</b>	<b>&lt; 0.001</b>	<b>F<sub>21</sub> &lt; F<sub>10</sub>, F<sub>32</sub></b>
	Treatment	0.349	0.910	-
Growth in tibia length	Sex	0.432	0.512	-
	<b>Larval instar</b>	<b>20.137</b>	<b>&lt; 0.001</b>	<b>F<sub>32</sub> &lt; F<sub>21</sub> &lt; F<sub>10</sub></b>
	Treatment	0.634	0.703	-

The adult traits did not change significantly according to the treatment in which individuals were reared (Table 3.5). However, larvae reared in the treatment D1 and D2 presented a slightly longer tibia than larvae reared in the control pond. Adult traits were significantly related to the sex of the individuals. The total length was higher in males than in females, whereas females had a larger head, longer wings and a higher body mass at emergence than males (Table 3.5).

**Table 3.5.** Results of two-way ANOVA and Scheirer-Ray-Hare tests performed on adult traits according to the sex and treatment. Values below the  $\alpha = 0.05$  threshold are printed in bold.

Response variable	Factor	Test	Statistic	P-val	Post-hoc test
Total length	<b>Sex</b>	Two-way	<b>5.148</b>	<b>0.026</b>	<b>M &gt; F</b>
	Treatment	ANOVA	0.553	0.792	-
Head width	<b>Sex</b>	Two-way	<b>8.670</b>	<b>0.004</b>	<b>F &gt; M</b>
	Treatment	ANOVA	0.193	0.986	-
Tibia length	Sex	Two-way	0.026	0.872	-
	Treatment	ANOVA	2.094	0.051	CP < D1, D2
Wing length	<b>Sex</b>	Scheirer-	<b>24.68</b>	<b>&lt; 0.001</b>	<b>F &gt; M</b>
	Treatment	Ray-Hare	9.028	0.473	-
Body mass	<b>Sex</b>	Scheirer-	<b>12.562</b>	<b>&lt; 0.001</b>	<b>F &gt; M</b>
	Treatment	Ray-Hare	7.470	0.280	-

The final GzLM model after backward step selection showed that the level of the Hsp70 protein was best predicted by the variable sex and treatment. The level of Hsp70 protein was significantly higher in adults than in larvae (Post-hoc p-value < 0.001; Fig. 3.3a) and significantly higher for the treatment D1 than for the treatment D2 (Post-hoc p-value = 0.024; Fig. 3.3b)



**Fig. 3.3.** Mean levels of Hsp70 protein according to the individual stage (a) and the treatment (b). The scale is an arcsin-square-root transformation on the proportion of protein optic density compared to the reference HeLa cell. Letters indicate the significance of post-hoc tests.

## 4. Discussion

Freshwater species inhabiting small waterbodies like ponds can have to face occasional pollution events during their development (Moss 2008). In this study larvae of *Aeshna cyanea* were exposed concentrations up to 30 mg.L<sup>-1</sup> of Roundup and DEET in water during their last larval stage. However, no increase in mortality, physiological stress or change in morphology could be detected in both larvae and adults. This study confirmed the general low sensitivity of odonates to water pollution (Oertli 2008).

Aquatic larvae exposed to pesticides often present a lower body mass resulting in a smaller size at emergence (Janssens et Stoks 2013; Tüzün & Stoks 2017). However, the presence of Roundup or DEET at concentrations up to 10-30 mg.L<sup>-1</sup> in the water did not have any effect on the larval growth or the traits at emergence in *Aeshna cyanea*. Moreover, the effects of pesticides are often enhanced by fluctuating or higher temperatures (Dinh et al. 2014; Arambourou & Stoks 2015;

Verheyen & Stoks 2020) and food deprivation (Janssens & Stoks 2013; Janssens et al. 2014). These environmental perturbations, even when they are delayed on time, can interact together and lead to and higher global effect on individual condition (Janssens et al. 2014). Therefore, individuals reared at high food level at a constant temperature may not always show physical differences in traits according to the treatment. In the present study larvae were fed ad libitum, which might have enabled them to cope with the expected effect of the water pollution. Moreover, the low and constant room temperature of our study (*i.e.* 17.2°C) might also have helped preventing from a potential stress caused by Roundup and DEET treatments.

The levels Hsp70 of insects are increasingly used as a biomarker because they are sensible to heat waves, but also many toxic chemicals that can be found in the water (Moreira-de-Sousa et al. 2018). In this study, larvae were exposed to a pesticide during at least one month and across several larval stages, but they had similar levels of Hsp70 according to the treatments. Only the individuals from the D1 treatment (*i.e.* DEET up to 30 mg.L<sup>-1</sup>) presented a level of Hsp70 significantly higher than those from the D2 treatment (*i.e.* DEET up to 30 mg.L<sup>-1</sup>). However, this result is hard to interpret since no difference was found with the individuals from the control pond or from the laboratory control. This global lack of response could be explained by the fact that Hsp70 level might have reached a peak at the beginning of the experiment and then returned to their basal level with time due to a process of stress habituation (McEwen 2007). A previous study on the damselfly *Ischnura elegans* was able to detect changes in protein levels with exposure to chlorpyrifos (2.0 µg.L<sup>-1</sup>), after a complete Fo development (Janssens et al. 2014). However, the changes in Hsp70 levels in *I. elegans* depended mostly on the temperature and food level, and only a small increase was attributed to the presence of pesticide. Overall, the lack of response of *A. cyanea* to water pollution in our study might be due to both, a high tolerance of the studied species and a long exposure time that enable the individuals to habituation.

Interestingly, the level of HSP70 was higher in the head of adults after emergence than in larvae and this, whatever the treatment. This result confirms that the complex processes of metamorphosis and emergence also cause a high stress to individuals (Jakob & Suhling 1999). This high stress due to emergence might have increases the inter-individual variability in Hsp70 levels and masked the potential stress caused by water pollutants in the studied adults.

Studies on a large number of organism have shown as a general rule that the metamorphosis was not a new beginning (Pechenik 2006). In the present study, several morphological traits of larvae

were strongly related to those of the adults. Especially, correlations were significant up to the larval stage F-3 for the length of hind tibia. Although the tibia length is not the easiest trait to measure on live larvae due to its small size, this morphological trait was very relevant in our study. Some previous studies on odonates already noticed that measurements of larval tibias were good predictors of adult body mass and size (Mikolajewski et al. 2004; Johansson et al. 2005). This relationship between leg size across metamorphosis may be stronger in Aeshnidae than in other odonate families such as Gomphidae, whose legs have a burrowing function at the larval stage (Leipelt et al. 2010). Despite their relevance, measurements of tibia length are seldom used in odonates trait studies and this trait is absent from both the “Odonate phenotypic database” (Waller et al. 2019) and the “Morphological and geographical traits of the British odonates” (Powney et al. 2014). However, this morphological trait might be a promising feature to consider in future work on carry-over effect in odonates.

#### ***Further work:***

The degradation of pollutants in water is a complex mechanism which depends on water composition and interactions with microbial communities (Lipok et al. 2010). For instance, the half-life of glyphosate can range from 8 to 120 days in pond water (Barolo 1993). To take this potential degradation of the chemical into account, water samples were collected each week during the experiment and stored at -20°C. A chemical dosage of these samples will assess the actual concentration of several chemical products (*e.g.* Glyphosate, POEA, AMPA, DEET) to which the larvae were exposed. This may allow a better understanding of the degradation of Roundup and DEET during the experiment. Information on the actual concentrations to which the larvae were exposed are also essential to provide a more accurate assessment on the actual tolerance of *A. cyanea* to water pollution.

## **5. Acknowledgements**

We would like to thank the laboratory Glyco-MEV for welcoming us and allowing to perform the Western Blots. Thanks are especially due to Carole Burel who spend time to teach us the method. We also thank Alexia Leveillé for her help in rearing the larvae and Sylvaine Buquet for her help during the laboratory work.



## Part III

# Dispersal of the large dragonfly *Anax imperator*

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## **Chapter 4**

### **Habitat use and movements of a large dragonfly (Odonata: *Anax imperator*) in a pond network.**

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#### **Abstract**

Local movements of aquatic insects within the surroundings of waterbodies aim essentially to find food, mates, resting sites or avoid predation. Distances moved are very variable among species and may also differ depending on sex or age at the intraspecific scale. Despite a large panel of studies on odonate activities near waterbodies, little is known about their movements and behaviour in the surrounding landscape matrix. This knowledge is however crucial to support management schemes of pond networks and allow improvement of connectivity between them.

In this study, we aimed to quantify movements at the landscape scale and investigate terrestrial habitat selection of the large dragonfly *Anax imperator* at the interface between a rural area and a suburban area in northwestern France using both mark-resighting and radio-tracking methods. A total of 87 individuals were identified with a unique code on the wings and monitored visually on five ponds during summers 2017 and 2018. Simultaneously, 54 individuals were equipped with radio-transmitters and tracked during up to 15 days.

A reduction of the survival rate was observed on the day immediately following capture regardless of the marking method. Individuals equipped with radio-transmitters had a lower estimated daily survival (0.78; 95% CI = 0.70–85) compared to wing-marked individuals (0.89; 95% CI = 0.85–0.92). Wing loading and age were the main parameters influencing dragonfly survival for both methods. The probability of movement between ponds was similar for both sexes, but radio-tracking data showed that females moved significantly further away in the landscape matrix than males, with a female notably detected up to 1902 m far from the release pond. Females also had a larger home range (mean 95% Kernel: 50 Ha) than males (mean 95% Kernel: 5 Ha). Reproductive behaviour of males and flying behaviour of females were positively related to air

temperature. Individuals of both sexes were significantly more present on the ponds than in all other habitats whatever their activity. They preferred significantly high trees as habitat to rest, when environmental temperatures were lower, especially females.

Overall, this study highlights the importance of integrating neighbouring trees in management schemes of ponds. It also confirms that preservation of pond networks must be conducted on several hectares around the ponds to encompass both aquatic and terrestrial requirements of freshwater organisms such as odonates. Our radio-tracking data provide a basis for further studies on the persistence of meta-populations in fragmented landscapes.

**Key words:** Dispersal, Home range, Mark-resighting, Telemetry, Survival

## 1. Introduction

At temperate latitudes, most aquatic insects have a long larval development and a short adult life expectancy (Cayrou & Céréghino, 2005). Since larvae cannot live away from the water, flying adults are the major dispersers of species inhabiting lentic waterbodies (Bilton et al., 2001; Lancaster & Downes, 2017). Long-distance flights are very rare at the individual scale, but these dispersal events have significant impacts on population maintenance by favouring colonisation and genetic diversity (Keller, 2010; Suhling et al., 2017). On the contrary, local movements are very frequent during adult life and aim essentially to find food, mates, resting sites or to avoid predation within the surroundings of waterbodies. Short-distance movements may also allow for step-by-step dispersal in connected landscapes such as dense pond networks (Coughlan et al. 2016).

Whether they represent dispersal events or local movements within the home range, distances moved by insects are very variable among species. They depend on intrinsic factors such as flight capability (Lancaster & Downes, 2017) and may differ according to age or sex at the intraspecific scale (Lancaster et al., 2020). These movements also depend on extrinsic factors such as land use, connectivity in the surrounding matrix (Maynou, 2017) and meteorological factors (*e.g.* temperature; May, 2019). Overall, understanding processes that shape movements and distribution at individual scale provides insights into population dynamics (Lowe & McPeck, 2014). Studies on animal movements are thus crucial to drive policies on management and conservation, in particular pond layout, land use, or habitat restoration (Allen & Singh, 2016; Peterman, 2018).

Odonates are insects with an aquatic larval stage and a flying imaginal stage. Adults are mostly found near waterbodies, where they come back after maturation (Corbet, 1999). Many species have particular habitat requirements to breed, rest and need a high food availability around the waterbodies (Chovanec & Waringer, 2001). Because of these requirements, odonates are often used as indicators of integrity and ecological functionality of lotic and lentic ecosystems (Chovanec et al., 2005; Simaika & Samways, 2009; Berquier et al., 2016). Adult dragonflies spend a great amount of their time foraging and resting in terrestrial habitats (Bried & Ervin, 2006). They can also fly away from waterbodies over long distances in order to find sites with lower competition or with higher food resources (Popova, 2017). However, most studies focused on activities of males near the ponds. Little is known about the requirements of both sexes in the surrounding terrestrial habitats (Dolný et al., 2014; Hykel et al., 2016; Le Naour et al., 2019) and their activity patterns in the landscape

(Hykel et al., 2018). Indeed, this information is difficult to obtain for most species because of their elusive behaviour when they are away from the ponds (Moskovitz & May, 2017; Hykel et al., 2018).

Apart from several tropical exceptions (Corbet, 1999; Feng et al., 2006), odonates are mainly active during the day (Jödicke, 1997; Hassall & Thompson, 2008; Borkenstein et al., 2017). Their behaviour is highly dependent on meteorological conditions. For small species, the wind often inhibits activity (Waringer, 1982), while temperature and sunshine are the main factors conditioning flying and reproduction (May, 1976; Hilfert-Rüppell, 1998; De Marco & Resende, 2002). For instance, the number of sunny days is determinant for survival and egg production of females (Thompson, 1990; Sherratt, 2010).

Wing marking is the most common method to study movements, abundance and habitat use of odonates, although higher mortality can be observed on the day just after marking (Cordero-Rivera & Stoks, 2008). Moreover, studying movements and habitat selection using wing marking requires a huge field effort to get enough resightings. Radio-tracking enables a large detection range but the weight of radio-transmitters is an important constraint that makes them suitable to track only some of the largest dragonfly species (May & Matthews, 2008). Previous studies using this method brought information about the migration of *Anax junius* on several hundreds of kilometres along the Eastern coast of the USA (Wikelsky et al., 2006; Knight et al., 2019) and enabled first quantifications of the home range for *Anax imperator* (Levett & Walls, 2011) and *Cordulegaster erronea* (Moskovitz & May, 2017).

The present study aimed to quantify local movements and habitat use of the large dragonfly *Anax imperator* within a pond network. This common species is the largest dragonfly in Western Europe (Dijkstra, 2015), making it a good candidate for carrying a radio-transmitter. Wing marking and radio-tracking were used independently to monitor individual movements between ponds and within their terrestrial surroundings. Distances travelled and home ranges were then estimated. Capture-Recapture models were fitted on the data to compare mortality rates between the two tracking methods and estimate the movement probabilities between the studied ponds. Effects of individual traits on survival probability were also investigated. A Step Selection Analysis (SSA) was performed on the telemetry data to identify the terrestrial habitats selected by *A. imperator*. Effects of the landscape context, individual traits and activity on habitat selection were also investigated in the SSA. Finally, the effect of meteorological conditions, and especially the ambient air temperature, on the behaviour of *A. imperator* was investigated.

## 2. Material and methods

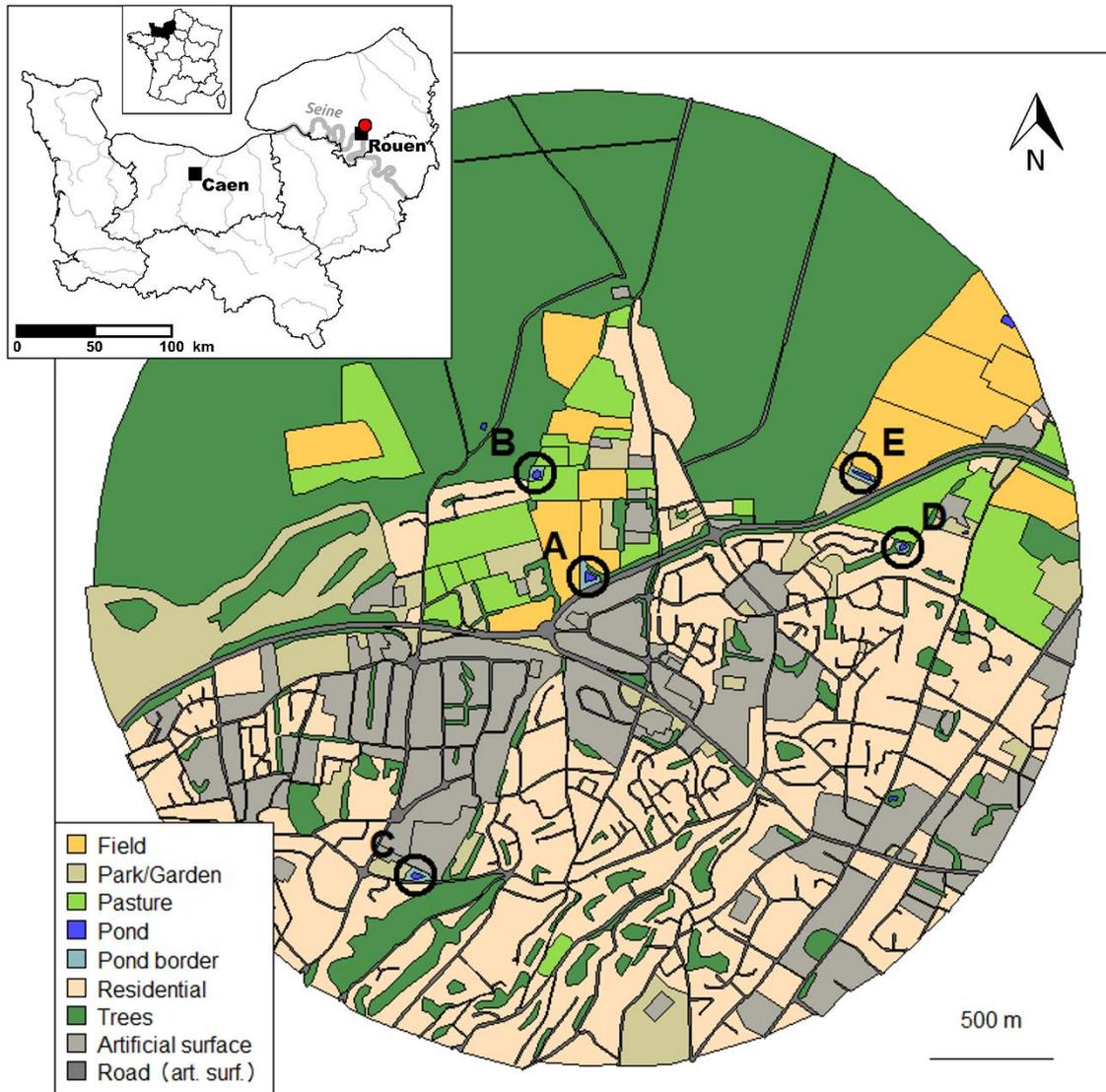
### 2.1. Study area

The study was conducted in the agglomeration of Rouen, Normandy, France (WGS84 coordinates: 49.480°N, 1.102°E). This area populated with 738 inhabitants per km<sup>2</sup> (insee.fr, 2015) is located along the Seine river, between Paris and the English Channel. The climate is oceanic temperate with a mean annual air temperature of 10.5°C and total annual precipitation averaging 851.7 mm (Météo France, 2020). Sampling was performed in 2017 and 2018 in the northern suburban area of Rouen, at the interface between urban activities and rural lands, separated by an East-West highway (Fig 1). The landscape consists mostly of residential neighbourhood and commercial areas to the south, while the northern part is essentially composed of a forest surrounding pastures and corn and wheat fields. Five large (*i.e.* 1016 ± 361 (SD) m<sup>2</sup>) and sun-exposed ponds were selected based on their attractiveness for *A. imperator* within a radius of 2 kilometres. They were located either in rural or in suburban context (Table 4.1). A weather station (Decagon EM50 DataLogger) equipped with thermometers (RT-1 Rugged Sensor), radiation sensors (PAR Photon Flux Sensor, Model QSO-S) and rain gauges (ECH2O Rain, Model ECRN-100) was installed both years on pond A. Meteorological data were measured continuously every minute and pooled by quarter-hour for the analyses.

### 2.2. Study species

The distribution of *Anax imperator* ranges from South Africa to Sweden. This species is the largest dragonfly in occidental Europe. It is commonly found on large, open and sun-exposed waterbodies with aquatic vegetation (Dijkstra, 2015). In the studied region, mature males measure in average 77.2 ± 2.3 (SD) mm in length with a mean body mass of 0.97 ± 0.07 (SD) g. Females are slightly smaller with a mean total length of 72.9 ± 2.3 (SD) mm and heavier with a mean body mass of 1.26 ± 0.09 (SD) g (Minot et al., 2019). Males always show a territorial behaviour on the pond, where they wait for females to breed from May to September. Larval development is expected to be univoltine or semi-voltine in the studied region and the maximum adult lifespan is about 60 days (Corbet, 1957). During the days following emergence, teneral odonates spend most of their active time foraging away from the ponds and seem to avoid all large reflecting surfaces (Corbet, 1957). Because of this elusive behaviour, they are difficult to find until their maturity, at which adults come

back to waterbodies to mate. In this study, only mature individuals caught near the ponds were marked, *i.e.* individuals with hard cuticle, hard wings and bright colours on the abdomen.



**Fig. 4.1.** Localisation of the study area in France (*i.e.* red circle) and detailed map of the eight habitat classes (see Table 4.S1 for details) on the area delimited by a 2 km radius around the pond A. Ponds A, B, C, D, and E used for the study are circled in black.

**Table 4.1.** Number of dragonflies equipped with radio-transmitters or wing-marked on each pond during the two sampling sessions in 2017 and 2018. Landscape context classification is based on Stamatiadis et al. (2017). The pond E, located in a ‘Rural’ landscape context, is not presented in the Table since no dragonfly was marked on this site.

Year	Landscape context (pond ID)	Radio-tracking		CMR	
		Females	Males	Females	Males
2017	Rural (A)	12	12	0	4
	Rural (B)	5	8	1	3
	Suburban (C)	0	2	0	2
	Suburban (D)	0	2	0	0
2018	Rural (A)	3	1	4	15
	Rural (B)	4	1	9	26
	Suburban (C)	4	0	4	12
	Suburban (D)	0	0	1	6
TOTAL		28	26	19	68

### 2.3. Marking protocol

Individuals were caught on the study ponds during CMR survey sessions or radio-tracking sessions using large aerial insect nets (*i.e.* frame size > 50 cm).

For the Capture-Mark-Resighting (CMR) study, individuals were marked with alphanumerical characters on wings using a permanent marker on fore-wings and white nail polish diluted to half strength with commercial acetone on hind-wings (Fig. 4.S1a). They were then identified through binoculars during subsequent sessions.

For the radio-tracking study, dragonflies were equipped with radio-transmitters (Advanced Telemetry Systems, model A2412; frequency range 148-152 MHz; 15 bpm; 0.2 g). Transmitters were placed on the ventral side of the thorax, posterior to the legs (Fig. 4.S1b). They were fixed using cyanoacrylate Krazy Glue and thin wound closure strips (Laboratoires Mercurochrome®) wrapped around the thorax. Mass of the equipment was on average  $25.2 \pm 1.9$  % (SD) of the insect’s body mass. These individuals were also marked with a code on the wing so that they could still be identified in case of transmitter failure. Radio-tracking was stopped when the transmitter was either lost, found on the ground or underwater. As dragonflies could unlikely detach transmitters on their own, transmitters found alone on the ground or underwater were considered as probable predation. In case the transmitters were found back after only a few days of tracking, they could be re-used to equip other dragonflies. A R410 scanning receiver (ATS) equipped with a 3 Element Folding Yagi

antenna was used to detect equipped dragonflies. Preliminary tests done on the field with the material showed a detection range of at least 200 meters in dense forests or residential areas, and of more than one kilometre through open areas.

Opportunistically, 4 individuals were also marked only on the wings during radio-tracking sessions. They were not equipped with radio-transmitters because their body mass was too light.

#### 2.4. Survey design

Fieldwork was conducted between 31 May and 7 September in 2017 and between 9 June and 22 August in 2018. All ponds were surveyed on the same days at least once a week, in order to record observations of all marked individuals. Visits were done between 10:00 and 17:00 only under favourable weather conditions (*i.e.* temperatures above 20°C by sunny weather or above 25°C by cloudy conditions). Pond banks and water surface were scanned using binocular during 15 to 30 minutes depending on the size of the pond. In 2017, ponds were surveyed 23 times, with a mean interval of  $4.3 \pm 5.6$  (SD) days between each visit. In 2018, the same ponds were surveyed 29 times, with a mean interval of  $2.6 \pm 2.1$  (SD) days between each visit.

Besides this work, 35 additional radio-tracking sessions in 2017 and 11 in 2018 were also conducted to allow catching, marking and tracking of individuals equipped with radio-transmitters only. Sessions occurred every day following attachment of radio-transmitters, independently of the weather conditions until the signal was definitively lost. Individuals were searched several times per day, at the location of the last contact and then making several concentric listening points within a 1 km radius around the last location until the signal was found. At each contact, time and position of the dragonflies equipped with transmitters were recorded. The behaviour of active individuals (*i.e.* flying or reproductive behaviour) and vegetation type on which individuals were resting (*i.e.* either high trees > 2m or low vegetation < 2m including small hedges and shrubs) were also reported. Reproductive behaviour included territorial males flying in circles above water and females laying eggs. No mating was observed during telemetry sessions. As it was sometimes difficult to distinguish whether individuals were hunting or not, this behaviour was simply reported as 'Flying'.

## 2.5. Traits measurements

Before marking, each dragonfly was photographed and put into a paper envelope in order to record its mass on an OHAUS Traveler TA152 scale with a precision of 0.01 g. Total body length and right hind wing length were also measured in the field using a GENERAL Metric Dial Caliper with a precision of 0.1 mm. Wing wear was evaluated on photographs and used as a proxy for age (McCauley, 2010). We defined a four-point scale as follows: (1) no wing wear, (2) a total of 1 to 30 cells missing, (3) more than 30 cells missing and (4) at least one wing broken proximal to the pterostigma. Individuals bearing obvious lesions on the body (*e.g.* broken frons or holes in the thoracic cuticle) were also considered in the 4th age category. Additionally, wing area of fore- and hindwings were measured using photographs of alive individuals in dorsal view with the software ImageJ (Schneider et al., 2012). Then, wing loading was calculated as the ratio of the body mass divided by the total wing area (Tüzün et al., 2018).

## 2.6. Data analysis

### *Capture-Mark-Resighting analyses*

Datasets were analysed using Multi-state models implemented in program MARK (Cooch & White, 2019), after building the input matrices with R 3.6.2 software (R Core Team, 2019) using the package RMark (Laake, 2013). These models are based on the Cormack Jolly Seber (CJS) method that allows to estimate the survival and resighting probabilities of a population or a group of individuals using live encounter histories (Lebreton et al., 1992). However, Multi-state models also allow to include several sites in the same analysis and estimate movement probabilities between them (Lebreton & Pradel, 2002). Multi-state models were used with both CMR and telemetry data to estimate movement probability of wing-marked dragonflies between ponds and to compare survival between CMR and telemetry.

Capture histories of the CMR and radio-tracking data from 2017 and 2018 were merged with a gap of 275 days separating them and grouped by sex and marking method. Only days on which all the 5 ponds were visited were kept, leading to the removal of 4 individuals that were marked on wing during radio-tracking sessions from the CMR data. Multi-state models were fitted to estimate daily survival probability ( $S$ ), resighting probability ( $p$ ) and movement probability ( $\Psi$ ). To investigate for a direct effect of the marking on survival probability, two 'survival periods' were distinguished in the

model:  $St=0-1$  (*i.e.* the interval between marking and the first day after marking) and  $St=1-\infty$  (*i.e.* all subsequent intervals from the first day after marking; Cordero-Rivera & Stoks, 2008). Probabilities of resighting by CMR method were fixed to zero for all days on which only telemetry was done, to avoid skewing the estimators. Conversely, as dragonflies equipped with radio-transmitters were always found when present within the detection range, probabilities of resighting were fixed to 1 for each radio-tracking session. CJS models were then built with daily apparent survival decomposed into  $\Phi_{t=0-1}$  and  $\Phi_{t=1-\infty}$ . The CMR data were assigned to different states depending on the pond on which the individuals were caught. Distances between ponds were then grouped into 2 classes:  $\leq 500$  m (*i.e.* between pond A-B or D-E) and  $> 500$  m (*i.e.* all other combinations of ponds). Individuals of the radio-tracking dataset were all assigned to a single state that was different from those of the CMR data.

Multi-state models were selected based on the corrected Akaike's Information Criterion (AICc), and only the ones with a  $\Delta AICc < 2$  from the best model were kept. Individual traits (*i.e.* age, body mass, body length, wing length and wing loading; Table 4.2) were added as covariates in the selected models. Given the considerable number of possible covariate combinations, we first fitted models with a single covariate and only the ones that improved AICc were subsequently combined in models in addition or interactions. Missing values of covariates were replaced by the mean values for each sex (Cooch & White, 2019). Final estimates were then obtained using model averaging with RMark. The goodness of fit was performed with the program U-CARE 2.3.4 (Choquet et al., 2009) using the global Multi-state test. Since the structure of the data did not enable to perform this test on the whole dataset, tests were performed separately for the CMR and the radio-tracking datasets. The  $\hat{c}$  (*i.e.* chi-square statistic divided by the number of degrees of freedom) were also calculated with U-CARE to check for overdispersion.

### *Home range and habitat selection*

All statistical analyses were performed using R 3.6.2 software (R Core Team, 2019).

Home range is commonly defined as the total use of space by an animal during a specific interval of its life (Powell & Michiell, 2012). Here, the study focused on the mature adult life of *A. imperator* and home range estimation was based on all available radio-tracking data collected within the study area. Estimations were computed with both MCP and Kernel methods using the R package 'adehabitatHR' (Calenge, 2006). The Minimum Convex Polygon (MCP) is the oldest and most widely used method for home range estimation (Stickel, 1954; Ford & Krumme, 1979). MCP was computed here for comparison purposes, but Kernel Density Estimation (Worton, 1989) was proven to be more

reliable (Seaman et al., 1999), especially for low sample sizes (Börger, 2006). We choose here to work with the 95% kernel isopleth because individuals were not continuously tracked and were expected to move sometimes far away around the recorded locations. Comparisons between sexes for the tracking duration, maximum distance moved, and home range were performed using Student's t-tests when normality and homoscedasticity of the data were confirmed, and otherwise using Mann-Whitney U tests.

In order to study habitat selection by *A. imperator*, the landscape matrix was mapped within a 2 km radius around pond A using the software QGIS 2.8 (QGIS Development Team, 2015). Based on aerial photographs, habitats were categorised into 8 classes (*i.e.* field, park or garden, pasture, pond, pond border, residential area, trees and artificial surface; Fig. 4.1; Table 4.S1). Habitat selection by *A. imperator* was studied based on the movements recorded during the radio-tracking sessions using a Step Selection Analysis (SSA; Avgar et al., 2016). All steps of duration over 15 minutes and longer than 5 meters were kept for this analysis. For each observed step, 99 random steps were created to simulate available movements around the starting location with the R package 'amt' (Signer et al. 2019). Distributions of turning angles and step lengths were beforehand compared between both sexes using Kolmogorov-Smirnov Tests. A 'von Mises' distribution was then used for random turning angles and random step lengths were generated under a gamma distribution based on all available observed steps. Thus, 263 strata of 1 observed step and 99 corresponding random steps were obtained. Habitat corresponding to the location at the end of each step was extracted from the map (Fig. 4.1) and added to the database for further analyses. For 210 random steps, the habitat was not used because the end location was not in the range of the studied map. Step Selection Functions (SSF) were built following Muff et al. (2019) with the R package 'glmmTMB' (Brooks et al., 2017). Given the large number of individuals in the dataset and the heterogeneity of the tracking durations, it was not possible to fit SSF for each individual independently. Therefore, mixed effect models including an individual random effect were performed to test for individual heterogeneity in the data (Muff et al., 2019). As random effects did not improve the final model ( $\Delta\text{WAIC} = 649$ ), only fixed SSF are presented here.

Since the tracking of individuals was not done continuously in the time, step lengths did not reflect the real distances moved. Hence, lengths and turning angles were used to generate the random steps, but not included as explanatory variables in the models. A first single effect SSF was fitted with 8 classes of habitat (see Fig. 4.1), independently from all other covariates. Based on the results, the initial 8 classes were then pooled into 4 habitat classes (*i.e.* Pond, Trees, Artificial surface

and Other use) to build models including following covariates: wing loading, sex, age, habitat, activity and temperature (Table 4.2). Data of solar radiation and temperature were highly correlated ( $\text{cor} = 0.76$ ), thus only the temperature was included in the model. Only five data points were collected under rainy conditions, this variable was therefore omitted. Since the only variable that change between observed and random steps was 'habitat', all covariates could only be tested for an interaction with habitat but not as a main effect. Additions of the covariates terms were then tested and the resulting models were ranked based on their AIC. For the best model, pairwise post-hoc tests with the Tukey p-value adjustment were performed on the least-square means of the estimates to discriminate the selection strength for each habitat (R package 'lsmeans'; Lenth, 2016).

**Table 4.2.** Details on the variables used in the analyses. Variable names are displayed with their abbreviations in the brackets when appropriate.

Variable name	Variable description	Unit / Classes
distance (dist)	distance classes	< 500 m; 500-2000 m
method (m)	marking method	CMR; Telemetry
length	total body length	mm
mass	body mass	g
wing	wing length	mm
wing loading (wl)	mass/wing surface	mg.mm <sup>-2</sup>
sex (s)	dragonfly sex	female; male
age	age categories based on wing wear	1; 2; 3 or 4
habitat	habitat classes	8 classes (see Fig. 4.1) <sup>a</sup>
activity	observed activity of the dragonfly	active; resting
temperature	temperature recorded by the weather station	°C
context	landscape context of the releasing pond	rural; suburban

<sup>a</sup> 'roads' displayed on the map Fig. 4.1 are included in the habitat 'artificial surface' in the analyses.

In addition, the effect of temperature, sex, age, wing loading and landscape context (Table 4.2) on each dragonfly behaviour separately (*i.e.* 'Resting in trees', 'Resting in low vegetation', 'Flying', 'Reproductive behaviour') was investigated using a binomial Generalized Linear Models (GLM). To test for an effect of the marking on dragonfly behaviour, a categorical variable grouping the time in two periods (*i.e.* the marking period ( $t = 0-1$ ) or all subsequent days ( $t > 1$ )) was also added in the models. The best model was chosen using a backward step selection based on the AIC.

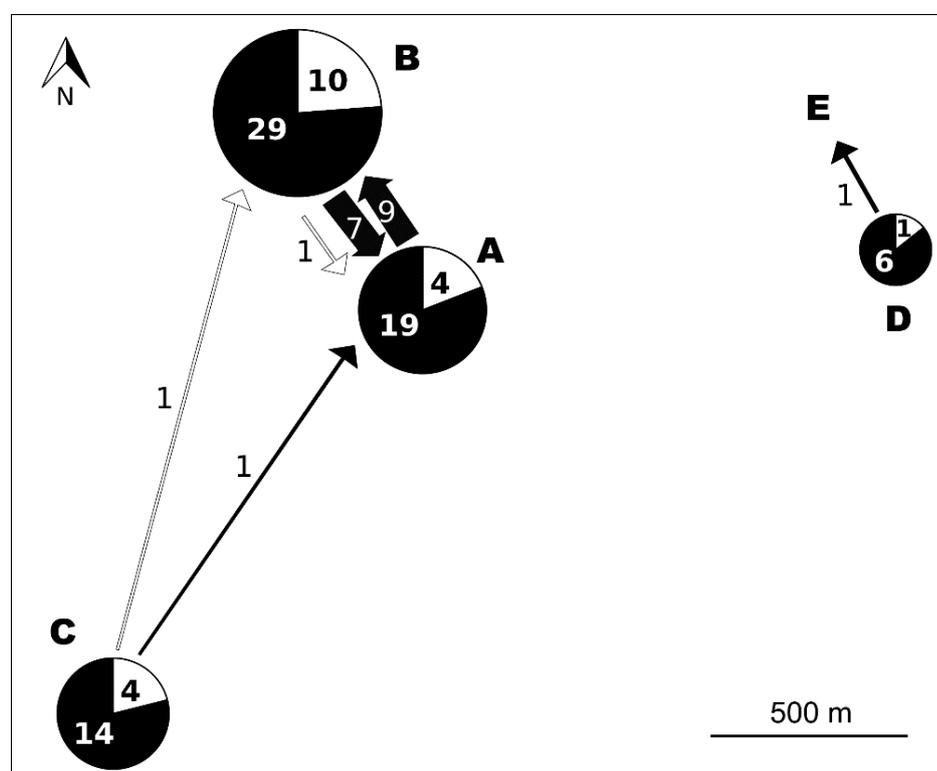
### 3. Results

#### 3.1. Survival and movements between ponds

Over both years, a total of 87 individuals were captured on ponds A, B, C and D and marked on the wings (Tables 1 & 3). Among them, 26% of the females and 45% of the males were resighted at least once. Fifteen individuals were seen away from their releasing pond at least once and 20 movements between ponds were recorded, with distance up to 1700 m by a female from pond C to B (Fig. 4.2).

**Table 4.3.** Values of mean and standard deviation (SD) of the total length, body mass and wing loading of the individuals used in this study.

	CMR		Telemetry	
	Female	Male	Female	Male
Total length (mm)	75.2 ± 1.9	78.2 ± 2.1	75.1 ± 1.6	79.1 ± 1.9
Body mass (g)	1.29 ± 0.15	0.98 ± 0.10	1.28 ± 0.10	1.01 ± 0.07
Wing loading (mg/mm <sup>2</sup> )	0.57 ± 0.05	0.44 ± 0.04	0.57 ± 0.04	0.47 ± 0.04



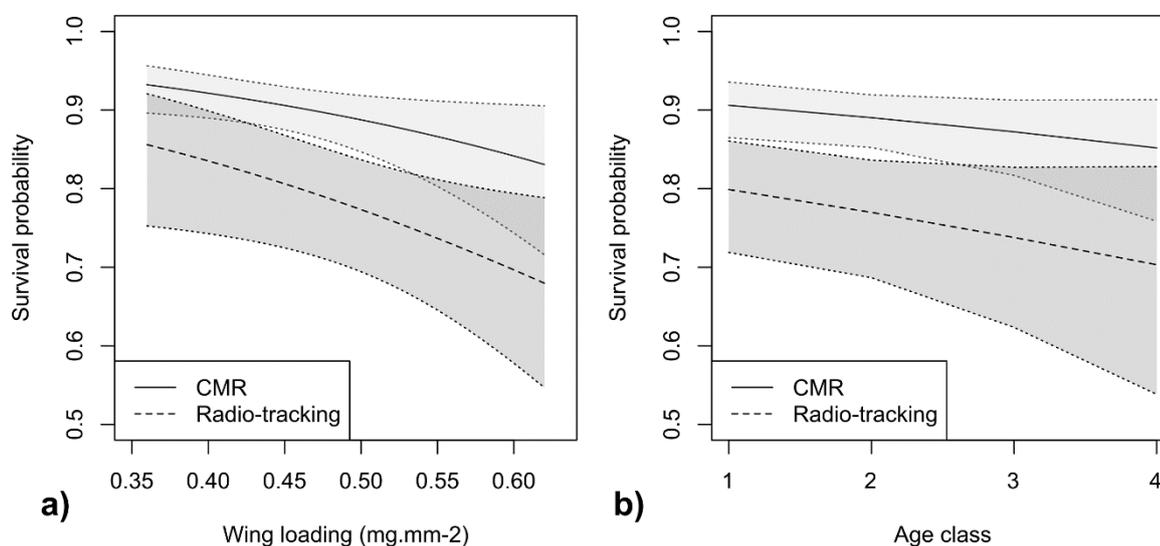
**Fig. 4.2.** Diagram map showing the sum of movements observed between ponds during the 2017 and 2018 CMR studies. Pies display the number of captured individuals on each pond (females in white and males in black). Number on arrows indicates the total count of movements for each sex. Pie size and arrow width are proportional to the number of marked dragonflies and movements.

Fifty-four dragonflies were equipped with radio-transmitters and tracked up to 15 days for females and 12 days for males (Tables 1 & 3). Twenty-four percent of the marked individuals were never found again after the day of marking. Then, 41% of the remaining individuals were lost between the first and the fourth day of tracking. For all individuals for tracked more than 4 days, radio-transmitters were retrieved after the end of the tracking (Fig. 4.S2). Overall, the reasons responsible for tracking ending were: 50% transmitters not found, 28% probable predation (*i.e.* transmitters found on the ground or underwater), 9% proved predation (*i.e.* observed or pieces of body found), 9% direct mortality (*i.e.* difficulties to take off and found dead on the day just after marking) and 4% transmitters out of order.

The final Multi-state models ranked by their AICc are shown in Table 4.4. The goodness of fit for the global multisite test with pooled sexes gave a p-value of 0.999 for the CMR group and a p-value of 0.988 for the telemetry group. Estimated  $\hat{c}$  were respectively 0.45 and 0.21, avoiding overdispersion correction. The probability of resighting was mostly dependent on the sex with  $pF = 0.20$  (95% CI = 0.10–0.47) for females and  $pM = 0.56$  (95% CI = 0.42–0.69) for males in the CMR group. The estimated daily survival  $St=1-\infty$  was 0.89 (95% CI = 0.85–0.92) for the CMR dataset and 0.78 (95% CI = 0.70–0.85) for the radio-tracking dataset. Survival was lower on the interval just after marking with  $St=0-1 = 0.76$  (95% CI = 0.66–0.83) for the CMR dataset and 0.56 (95% CI = 0.46–0.70) for the telemetry dataset. Daily estimated probabilities of movements were  $\Psi_{<500m} = 0.14$  (95% CI = 0.08–0.23) and  $\Psi_{500-2000m} = 0.003$  (95% CI = 0.001–0.012). According to the set of selected models, survival probability (S) was mostly negatively related to wing loading (Fig. 4.3a) and age at first capture (Fig. 4.3b). It was also positively related to wing length in one model. On the contrary, the probability of resighting (p) was positively related to wing loading, age and body mass. The probability of movement ( $\psi$ ) was negatively related to wing loading, age or body length in several models (Table 4.4).

**Table 4.4.** Best Multi-state models fitted with CMR and radio-tracking data of 2017 and 2018 grouped by tracking method ( $m$ ), and sex ( $s$ ). Model names are denoted by the daily survival ( $S$ ), resighting probability ( $p$ ) and movement ( $\Psi$ ). Variable names are: distance ( $dist$ ), time ( $t$ ), age, total length ( $length$ ), hind wing length ( $wing$ ), total body mass ( $mass$ ) and wing loading ( $wl$ ; details in Table 4.2). Time ( $t$ ) refers to the two periods:  $t_{0-1}$  and  $t_{1-\infty}$ . Columns list the model names, the corrected Aikaike information criterion (AICc), the difference from the best model ( $\Delta AICc$ ), the corrected Aikaike information criterion weights (AICc W), the model likelihood, the model deviance, and the number of parameters ( $K$ ). Only models which  $\Delta AICc < 2$  are presented here.

Model	AICc	$\Delta AICc$	AICc W	Deviance	K
$S(m + t + wl) p(s) \Psi(dist)$	720.14	0.00	0.08	701.59	9
$S(m + t + wl) p(s + wl) \Psi(dist)$	720.36	0.22	0.07	699.69	10
$S(m + t + wl + age) p(s) \Psi(dist)$	720.37	0.23	0.07	699.70	10
$S(m + t + wl + age) p(s + wl) \Psi(dist)$	720.52	0.38	0.06	697.72	11
$S(m + t + wl + age) p(s + age) \Psi(dist)$	720.63	0.50	0.06	697.83	11
$S(m + t + wl) p(s + age) \Psi(dist)$	721.04	0.91	0.05	700.38	10
$S(m + t + wl) p(s + age) \Psi(dist)$	721.04	0.91	0.05	700.38	10
$S(m + t + wl) p(s * wl) \Psi(dist)$	721.09	0.96	0.05	698.29	11
$S(m + t + wl) p(s + mass) \Psi(dist)$	721.10	0.97	0.05	700.44	10
$S(m + t + wl) p(s) \Psi(dist + age)$	721.28	1.14	0.04	700.62	10
$S(m + t + wl + age) p(s + mass) \Psi(dist)$	721.36	1.22	0.04	698.56	11
$S(m + t + wl + wing) p(s) \Psi(dist)$	721.39	1.26	0.04	700.73	10
$S(m + t + wl) p(s) \Psi(dist + length)$	721.68	1.55	0.04	701.02	10
$S(m + t + wl + Sex) p(s) \Psi(dist)$	721.81	1.67	0.03	701.14	10
$S(m + t + wl) p(s) \Psi(dist + wl)$	721.94	1.80	0.03	701.28	10



**Fig. 4.3.** Graphs showing the survival curves and 95% confidence intervals against individual covariates extracted from the Multi-state models. Solid lines represent the CMR group and dashed lines the radio-tracking group. Estimates for survival (i.e.  $\Phi_{t=1-\infty}$ ) against wing loading (a) are extracted from the model  $S(m + t + wl) p(s) \Psi(dist)$  in which apparent survival ( $\Phi$ ) depends on the marking method ( $m$ ), the time ( $t$ ), and wing loading ( $wl$ ); the probability of capture ( $p$ ) depends on the sex ( $s$ ); the probability of movement ( $\Psi$ ) depends on the distance. Estimates for survival against age class (b) are extracted from the model  $S(m + t + wl + age) p(s) \Psi(dist)$  in which apparent survival ( $\Phi$ ) depends on the marking method ( $m$ ), the time ( $t$ ), and age; the probability of capture ( $p$ ) depends on the sex ( $s$ ); the probability of movement ( $\Psi$ ) depends on the distance.

### 3.2. *Anax imperator's* home range

Based on the 15 individuals monitored over at least 24h with at least 5 locations, no significant difference in tracking duration was found between both sexes (Table 4.5). Distance moved from the release pond to the most distant recorded location was significantly higher for females (maximum = 1902 m) than males (maximum = 534 m; Table 4.5). One transmitter was found by chance about 5 km away to the North from its releasing pond. However, the antenna was severely bent on its whole length and no dragonfly remnants were found. As this transmitter was found beyond the limits of our study range and might have been brought by a bird, the data for this male was excluded from the analyses.

The MCP provided home range values from 5.22 Ha to 110.81 Ha for females and from 0.83 Ha to 12.57 Ha for males. The 95 % Kernel method gave higher values ranging from 4.08 to 196.88 Ha for females and from 0.78 to 14.34 Ha for males. Although there was a large variability between individuals, females had a significantly larger home range than males in all estimation methods (all  $p$ -values < 0.05, see Table 4.5).

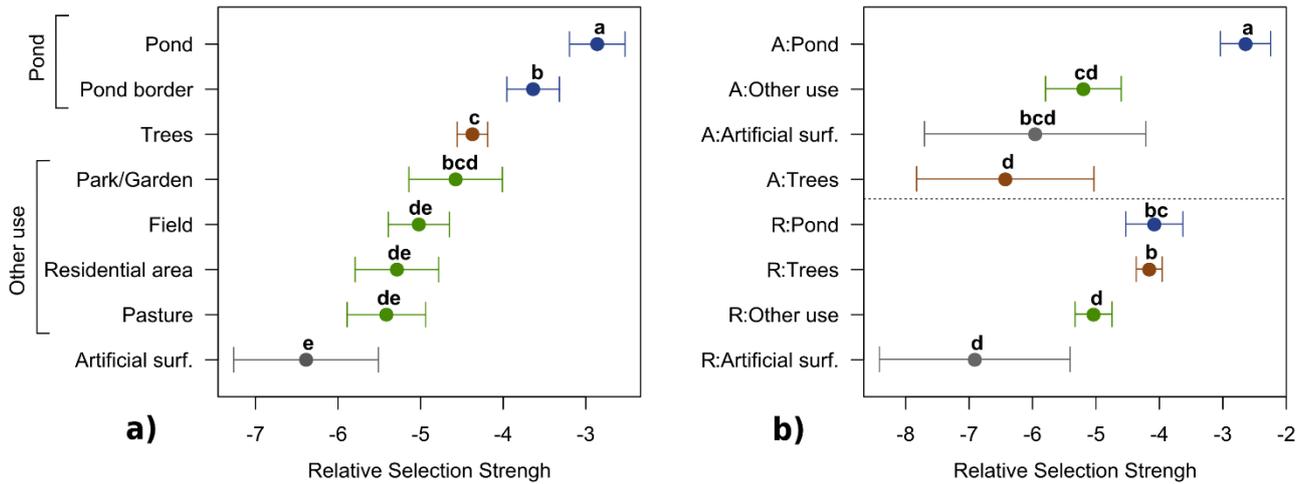
**Table 4.5.** Results of the radio-tracking study for individuals with a minimum of 5 points and tracking duration > 24 hours. Values are the means and standard deviations (SD) for: the duration between transmitter attachment and the last observation of the dragonfly alive; distance from the releasing pond to the most distant location recorded; home range estimated via the Minimum Convex Polygon method; home range estimated via the 95% Kernel method.  $P$ -values below the threshold  $\alpha = 0.05$  were printed in bold letters. Data of one transmitter found 5 km away from the release pond was not included in these results.

	n	Mean duration (days)	Mean distance (m)	Mean MCP (Ha)	Mean 95% Kernel (Ha)
<b>Females</b>	7	5.5 ± 4.5	814 ± 616	36.7 ± 44.0	51.5 ± 69.9
<b>Males</b>	8	5.4 ± 3.5	155 ± 100	4.0 ± 3.7	5.0 ± 4.3
<b>Statistic</b>	-	Mann-Whitney U Test; W = 26, $p$ -value = 0.866	Student's T Test; T = 2.80, $p$ -value = <b>0.029</b>	Mann-Whitney U Test; W = 53, $p$ -value = <b>0.002</b>	Mann-Whitney U Test; W = 49, $p$ -value = <b>0.014</b>

### 3.3. Habitat selection and behaviour of *Anax imperator*

No significant difference between males and females was found in the distribution of turning angles (Kolmogorov-Smirnov test,  $D = 0.097$ ,  $p\text{-value} = 0.574$ ), but the distributions of step lengths differed significantly (Kolmogorov-Smirnov test,  $D = 0.187$ ,  $p\text{-value} = 0.021$ ). Therefore, random steps were generated from these distributions separately for males and females. The first SSA done on eight habitat classes showed a strong tropism of dragonflies for ponds ( $-2.86 \pm 0.17$  SE) and their border ( $-3.64 \pm 0.16$  SE) compared to random steps (Fig. 4.4a). These two habitat classes were significantly different from all other classes (Tukey post-hoc test: all  $p\text{-values} > 0.05$ ). Relative selection strength estimate for trees ( $-4.37 \pm 0.09$  SE) was not significantly different from parks and gardens ( $-4.58 \pm 0.29$  SE; Tukey post-hoc test:  $p\text{-values} > 0.05$ ), but was significantly higher than for fields ( $-5.02 \pm 0.19$  SE), residential areas ( $-5.29 \pm 0.26$  SE), pastures ( $-5.42 \pm 0.24$  SE) and artificial surfaces ( $-6.39 \pm 0.45$  SE; Tukey post-hoc test: all  $p\text{-values} < 0.05$ ). Estimates for parks and gardens, fields, residential areas, pastures and artificial areas did not differ significantly from each other (Tukey post-hoc test: all  $p\text{-values} > 0.05$ ).

Since their estimates were very similar, parks and gardens, fields, residential areas and pastures were grouped in one habitat class named 'Other use', in the second SSA. Only the variable 'artificial surface' was not grouped in 'Other use', while it was not significantly different, because its estimate was very low compared to the others. Interaction of all covariates with activity were also tested during the step selection. The final model selected included the variable grouping the 4 habitat classes and its interaction with dragonfly activity classes and with temperature (Table 4.6). In this model, the variable temperature increased significantly the model's AIC, but its effect was not significant. There was a relationship between dragonfly activity and habitat selection. Resting dragonflies were significantly more found in ponds ( $-4.08 \pm 0.23$  SE) and trees ( $-4.16 \pm 0.10$  SE), than in 'Other use' habitats ( $-5.03 \pm 0.15$  SE) and artificial habitats ( $6.91 \pm 0.76$  SE; Tukey post-hoc test: all  $p\text{-values} < 0.05$ ). On the contrary, active dragonflies significantly preferred ponds ( $-2.64 \pm 0.20$  SE), but no significant difference was found between trees ( $-6.43 \pm 0.71$  SE), artificial habitats ( $-5.96 \pm 0.8$  SE) and 'Other use' habitats ( $-5.20 \pm 0.30$  SE; Tukey post-hoc test: all  $p\text{-values} > 0.05$ ; Fig 4b).

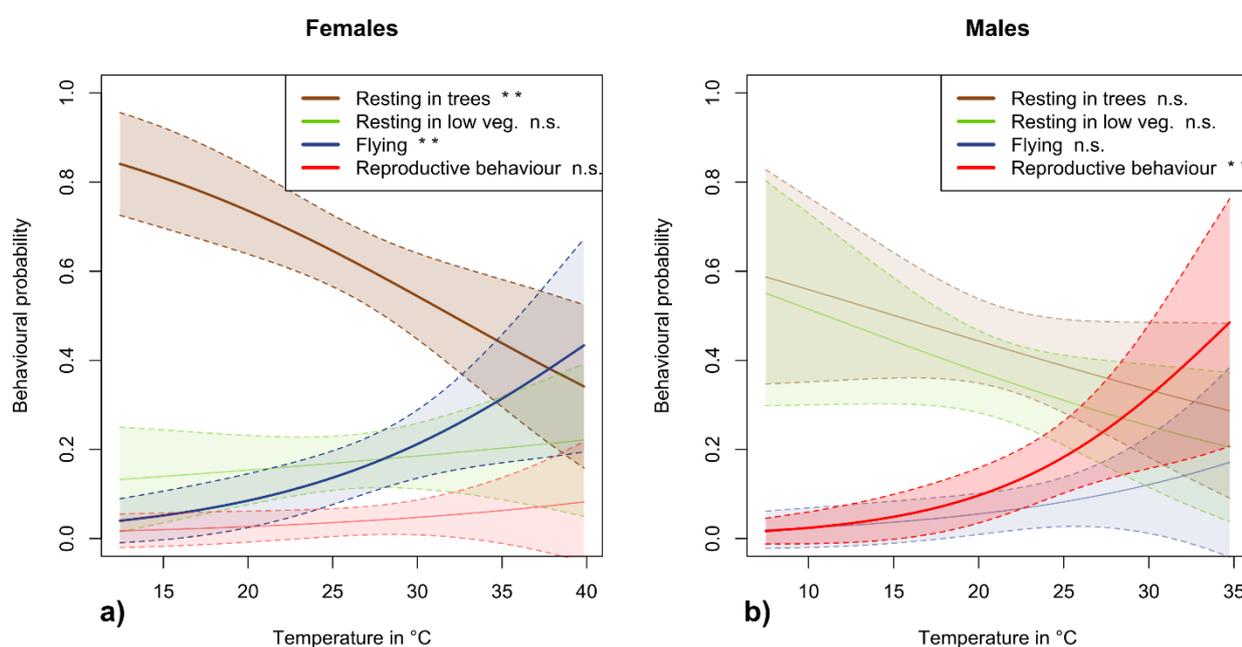


**Fig. 4.4.** Estimates for the relative selection strength with 95% confidence intervals. The eight classes from the first single effect model (a) were then grouped to 4 classes, according to the displayed colours. Plot (b) shows the results of the model of habitat selection and its interaction with dragonfly’s activity (i.e. A: active, R: resting) at the end of the step.

**Table 4.6.** Full models including 4 habitat classes and interaction with covariates. Models are ranked based on Akaike Information Criterion (AIC).  $\Delta$ AIC is the difference between each tested model and the best model retained printed in bold letters. Values of the variables ‘context’, ‘activity’ and ‘temperature’ are given for the end of the steps.

Model	$\Delta$ AIC	AIC
~ habitat + habitat:activity + habitat:temp	0.0	2155.0
~ habitat + habitat:activity	-210.8	2365.8
~ habitat + habitat:temp	-374.8	2529.8
~ <b>habitat (null model)</b>	<b>-641.0</b>	<b>2796.0</b>
~ habitat + habitat:wl	-645.5	2800.5
~ habitat + habitat:sex	-646.0	2801.0
~ habitat + habitat:context	-646.6	2801.6
~ habitat + habitat:age	-646.8	2801.8

GLMs showed that the probability of exhibiting reproductive behaviour was significantly positively related to air temperature ( $Z = 3.40$ ,  $p$ -value  $< 0.001$ ), age ( $Z = 2.62$ ,  $p$ -value =  $0.009$ ) and was negatively related to wing loading ( $Z = -3.26$ ,  $p$ -value =  $0.001$ ) and sex ( $Z = -2.26$ ,  $p$ -value =  $0.028$ ), while the day of marking had no significant effect ( $Z = -1.73$ ,  $p$ -value =  $0.083$ ). Especially, the reproductive behaviour probability of males was significantly positively related to air temperature (Fig. 4.5a), whereas there was no relationship between reproductive behaviour of females and air temperature (Fig. 4.5b). Flying behaviour was significantly positively related to air temperature ( $Z = 3.32$ ,  $p$ -value  $< 0.001$ ) and age ( $Z = 3.64$ ,  $p$ -value  $< 0.001$ ), but did not differ between sexes ( $Z = -1.92$ ,  $p$ -value =  $0.055$ ). The probability of finding a dragonfly resting in low vegetation increased significantly with the wing loading ( $Z = 2.88$ ,  $p$ -value =  $0.004$ ) and depended significantly on the sex ( $Z = 3.98$ ,  $p$ -value  $< 0.001$ ), with males being more often in low vegetation than females. The probability of finding a dragonfly resting in trees was significantly negatively related to air temperature ( $Z = -3.48$ ,  $p$ -value  $< 0.001$ ), age ( $Z = -3.15$ ,  $p$ -value =  $0.002$ ), day of marking ( $Z = -2.12$ ,  $p$ -value =  $0.034$ ), and differed between sexes ( $Z = -3.13$ ,  $p$ -value =  $0.002$ ). Only females significantly tended to rest in trees when air temperature decreased (Fig.5b).

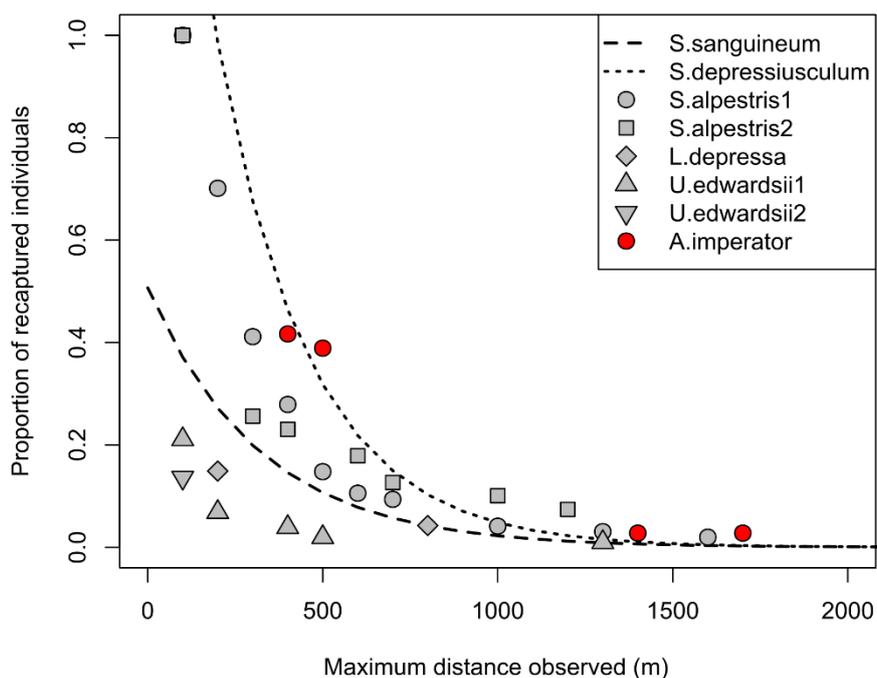


**Fig. 4.5.** Predicted behaviours and their 95% confidence intervals against ambient temperature at the time of the contact for females (a) and males (b). Asterisks in the legend show statistical significance of estimated coefficients for each behaviour: n.s. not significant; \*  $p$ -value  $< 0.05$ ; \*\*  $p$ -value  $< 0.01$ .

## 4. Discussion

Knowledge on odonate habitat preferences and movement patterns is determinant to support management schemes of ponds and improve connectivity between them (Raebel et al., 2012; Maynou et al., 2017; Khelifa, 2019). Females were already known to travel further distance from their natal sites than males (Dolný et al., 2014). However, differences in home range size were never tested in previous radio-tracking studies on odonates since they mostly focused on males (Levett & Walls, 2011; Moskowitz & May, 2017). This study highlighted several movements of *Anax imperator* between studied ponds and showed that females use significantly larger home ranges compared to males during their mature lifetime.

No movement was observed between the group of ponds A and B and the group D and E although they were only ca. 1 km apart. Since only a few individuals were marked on D and E, departures from these ponds might have been missed. They might also be less attractive than ponds A or B, preventing individuals to move in there. The daily probability of movement up to 500 m away was estimated at about 14% for both sexes. However, this estimate only represents the probability to move from one specific pond to another. The cumulative daily probability to move from the initial site to any other site in the surrounding up to 500 m is thus expected to be much higher for *A. imperator* when several ponds are available in the surroundings. Cumulatively over several days, this result suggests that movements between nearby ponds are frequent. No similar data on daily movements of Anisoptera is available in the literature for comparison. Some individuals also exhibited long-distance movements and the proportion of dragonflies that moved between ponds in this study is among the highest compared to the ones obtained in previous CMR studies (Fig. 4.6 & Table 4.S2). Several movements further than 1 km within a few hours were recorded during the radio-tracking survey, confirming previous results on the high mobility of this large species (Levett & Walls, 2011). Moreover, many individuals were lost, suggesting that they did probably undertake a long flight out of the detection range and never came back. Signal losses mostly occurred during the first few days of tracking. After four days, remaining dragonflies were never lost (*i.e.* they did not leave the studied area) and observed mortality was most likely due to predation. Therefore, we can hypothesize that some dragonflies spend all their adult lifetime in the same home range, while others disperse further away.



**Fig. 4.6.** Proportion of recaptured individuals in relation to the maximum distance they moved. Displayed regression curves are based on negative exponential functions and red points represent the results from the present CMR study. Details and source of the data used are available in Table 4.S2.

When studying local movements of a species, considering only distances moved do not always allow to distinguish dispersal events from local movements in the habitats. Clobert et al. (2001) defined the breeding dispersal as a ‘movement between two successive breeding areas or social groups’. Therefore, dispersal events among populations that lead to genetic exchanges might be considered as dispersal, while frequent movements between habitats that are effectively part of the study patches, might not be considered in the same way. In this study, daily movements were observed between ponds separated by 400-500 m, suggesting that *A. imperator* can probably use several ponds within its home range in order to find a variety of habitat types and breeding sites or to avoid intraspecific competition. Since studied ponds were sufficiently distant to be considered as distinct breeding areas, even these short movements between two ponds might be considered as dispersal events. Such movements, even at the scale of the home range, promote a good functional connectivity in the pond network and can contribute to gene flow (Matos et al., 2019). It underlines that dispersal events can occur at the scale of the home range depending on both the presence of several breeding sites in this home range and the sufficient time duration of the tracking to detect movements between them.

In this study, females were more mobile than males in terrestrial habitats. They probably move further away from the ponds to avoid sexual harassment (Corbet, 1999) and find more

resources to support egg development (Anholt et al., 1991). As they are in charge of egg production, females are also probably the limiting factor for population renewal. Providing areas around the ponds with places for females to roost such as trees and vegetation attracting other insects to feed would be a good way to increase population capacity of the ponds (Davies et al., 2009; Hykel et al., 2019). On the contrary, males have a territorial behaviour resulting in a smaller home range compared to females. Some of them establish as residents and defend lastingly their territory (Higashi, 1969; Koenig & Albano, 1987; Kasuya et al., 1997). The others, called itinerants, will leave the pond to find another one (Lancaster & Downes, 2017) in their home range or further away. Overall, the landscape characteristics (*e.g.* habitats and food availability) on several hectares around the waterbodies and the connectivity in a patchy environment seem of high significance for foraging activities and roosting for both sexes. This confirms a previous study showing that the terrestrial home range of a dragonfly could be about 1000 times greater than the natal waterbody in which larval development occurred (Dolny et al., 2014).

Previous work on Anisoptera reported a diel change in habitat use and especially, an avoidance of ponds during the night (Hykel, 2018). In most species, males come to the ponds at the hottest periods of the day and wait for females (Suhonen et al., 2008). As expected, active dragonflies showed a strong tropism for ponds and their borders in this study. The probability of flying or showing a reproductive behaviour near the ponds was positively related to ambient temperature and age. This result is also consistent with the mark-resighting study, in which resighting rates near the ponds were higher for older individuals. Temperature and age were also negatively related to the resting behaviour in high trees, especially for the younger females. In Northern Europe, the presence of trees near the shoreline was shown to positively influence odonate species richness (Sahlén, 2006). However, tree shading on ponds is also often associated with a loss of macrophyte cover and a decrease in freshwater species richness (Sayer et al., 2012). At individual scale, this study showed that resting dragonflies, especially females, selected high trees more than other available habitats of the surrounding matrix. Indeed, trees can provide reliable roosting sites during the reproductive period to survive severe weather conditions and to avoid predation. It complements a recent study highlighting the role of forest canopy as a preferential maturation habitat for an endangered dragonfly (Le Naour et al., 2019).

Resighting probabilities were rather high for both sexes compared to previous CMR studies on other anisopteran species (Table 4.7). *Anax imperator* is a large Aeshnid species, making it probably easier to detect on the ponds than other Anisopteran species. As expected from the

literature on mark-resighting on odonates (Cordero-Rivera & Stoks, 2008), daily resighting rates were higher for males (0.56) than for females (0.20), due to the fact that males spend more time near water waiting for a mate. Despite these relatively high resighting rates, many individuals were never resighted on successive days. Several reasons can explain this observation such as the temporary absence of the marked individuals on the pond at the time of the sampling session, their final departure from the pond and its surroundings or their death. Thus, only marking a large number of individuals enables to detect movements between ponds by this method.

**Table 4.7.** Published values of recapture rates for mature males and females from previous mark-resighting studies on odonates. Number is the total number of individuals marked in the study.

Species	Number	Male	Female	Reference
<i>Aeshna umbrosa</i>	48	18%	20%	Halverson, 1984
<i>Aeshna tuberculifera</i>	52	27%	27%	Halverson, 1984
<i>Cordulia shurtleffii</i>	365	17%	-	Hilten, 1983
<i>Erythrodiplax umbrata</i>	632	18%	14%	Palacino-Rodriguez et al., 2012
<i>Leucorrhinia caudalis</i>	516	25%	7%	Keller et al., 2010
<i>Libellula depressa</i>	117	49%	29%	Angelibert & Giani, 2003
<i>Somatochlora alpestris</i>	314	59%	29%	Knaus & Wildermuth, 2002
<i>Sympetrum danae</i>	1561	23%	9%	Michiels & Dhont, 1991
<i>Sympetrum depressiusculum</i>	3001	1%	1%	Šigutová et al., 2017
<i>Sympetrum infuscatum</i>	5480	1%	-	Watanabe et al., 2004
<i>Anax imperator</i>	87	43%	26%	Minot et al. (present data)

None of the previous studies on odonates assessed or reported an effect of transmitters on survival (e.g. Wikelsky et al. 2006; Moskowitz & May, 2017). However, the radio-transmitter load might reduce flight agility, making them more vulnerable to frog and bird predation. Here, a comparison between radio-tracking and wing-marking demonstrated a lower survival rate of individuals equipped with radio-transmitters. The ratio of the transmitter weight on *Anax* body mass (25%) was about the same as in previous studies on odonates (e.g. Wikelsky et al. 2006; Levett & Walls, 2011, Moskowitz & May, 2017). This percentage is very high compared to the ratio (i.e. < 5 %) recommended for bird and bat (Kissling et al., 2014). However, flying insects seem able to carry heavier loadings, sometimes exceeding a third of their body mass (e.g. 35% in *Xylocopa flavorufa*; Pasquet et al. 2008). The transmitter weight did not seem to impact all individuals in the same way. While some dragonflies rested more than half an hour or could hardly take off after transmitter attachment, several other individuals were followed more than one week, showing reproductive behaviours. Interestingly, in both methods, our results suggest that survival does not depend on

individual body mass, but rather on age and wing loading. Thus, large wing size and low mass seem to be more relevant to consider than only high body mass when selecting the dragonflies to equip with radio-transmitters. Yet selecting such individuals may generate a bias in observed dispersal as, in many animal species, dispersal propensity or capacity is affected by morphological characteristics (Lowe & McPeck, 2014).

With both marking methods, a decline of the estimated survival was highlighted on the day following the capture. This peak of apparent mortality is more likely due to the stress of the capture and the manipulation, than a direct effect of the marking (Cordero, 1994), since no physical harm was recorded during the marking process. Individuals followed by telemetry did not significantly show an avoidance of the ponds or move to other ponds just after marking. However, direct mortality and many departures from the study area were observed in dragonflies equipped with radio-transmitters on the day of marking and the first day after (Fig. 4.S2). Even if radio-tracking is restricted to large odonate species and can have an impact on survival, this method brings new insights on dragonfly behaviour and movements in their terrestrial habitats, by recording precisely individual locations independently of their sex and even by night or unfavourable weather conditions. Especially, this method allowed us to find dragonflies when they were hidden in the matrix landscape, for instance, high in the tree canopy. This study suggests that the effect of the transmitter weight can be reduced by choosing individuals according to their age and wing loading to ensure better survival and longer tracking duration with the transmitters.

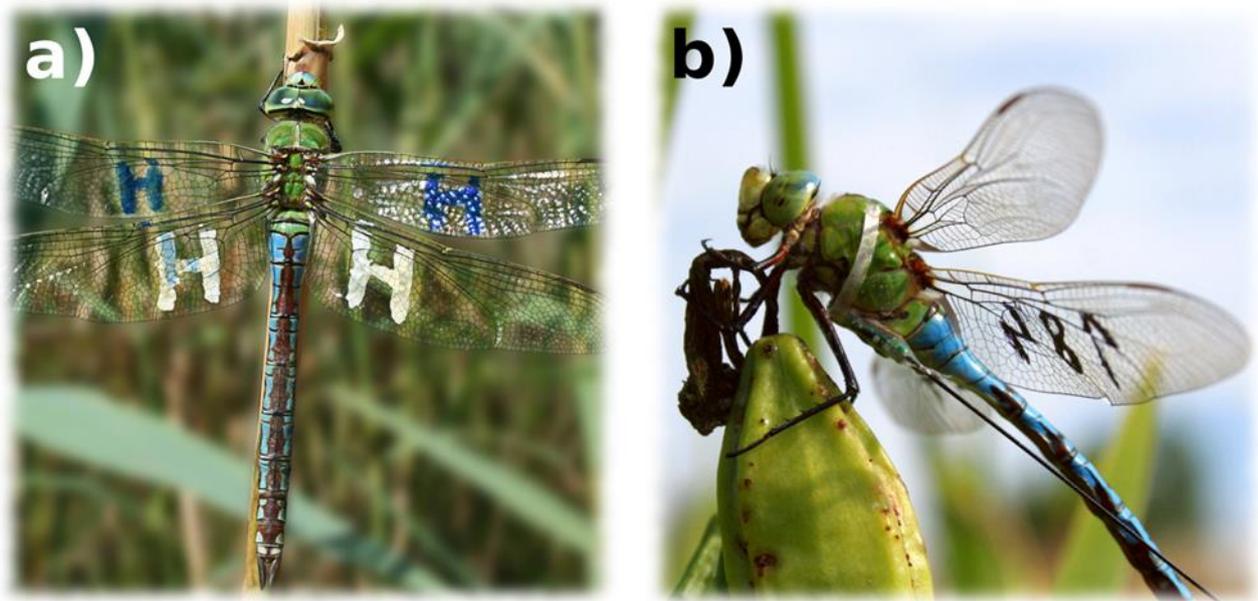
Some individuals are able to fly long distances and have large home ranges, making it sometimes difficult to distinguish prospecting movements within the home range from dispersal (Van Dyck & Baguette, 2005). Large dragonflies can use several ponds across fragmented landscapes during their lifetime, highlighting the importance to consider ponds not as single units, but as connected in a network (Maynou et al., 2017). The connectivity between ponds depends on the composition of the terrestrial matrix and was already shown to have a strong impact individual on survival of other freshwater species (Owen-Jones et al., 2016; Cayuela et al., 2019; Serrano et al., 2020). This study on *Anax imperator* provided an example of local movements, home range and terrestrial habitat requirements of a large dragonfly species. The presence of sparse trees, near pond banks or the proximity of a forest, must be taken into account for the management of ponds and their surroundings to favour presence of adults. Moreover, freshwater species conservation must also consider aquatic larval stage for which biological and physical characteristics of the ponds are determinant. Especially, the presence of predators such as fishes can impact negatively local

population maintenance and structure of freshwater communities (Smith, 1999; Stoks & McPeck, 2003), whereas emergent vegetation are important habitats providing food resource, refuge and support for eggs (Hartel et al., 2007; Huikkonen et al., 2019). Overall, our radio-tracking data contribute to highlight the terrestrial requirements of a freshwater species on a large spatial scale and provide a basis to support further network-based models on meta-population persistence (Hanski & Ovaskainen, 2000; Zamberletti et al., 2018).

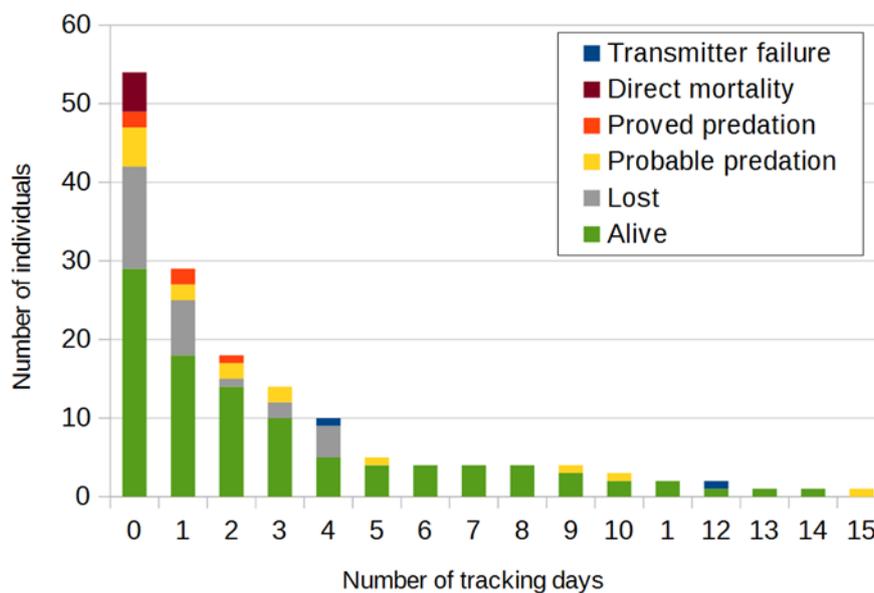
## 5. Acknowledgements

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## 6. Supplementary materials



**Fig. 4.S1.** Illustration of the two marking methods on *A. imperator*: a) dragonfly only marked on the wings using white nail polish and permanent marker; this female 'H' was seen again over 1 km away from its releasing pond; b) radio-transmitter glued below the thorax of the dragonfly and frequency written on the hind wings with a permanent marker; this male '184' was followed during 14 days around pond A.



**Fig. 4.S2.** Reasons for tracking ending: number of individuals for each status depending on the duration of tracking.

Table 4.S1: Details of the 8 classes used to characterize terrestrial habitats of the map presented on Fig. 4.1.

Name of the habitat class	Description
Artificial surfaces	Areas dominated by large buildings, sealed surfaces, or bare surfaces without high vegetation ( <i>e.g. parking, sport courts</i> ), including all sealed roads which width > 4m.
Field	Arable land used to grow crops ( <i>e.g. wheat, corn</i> ).
Pasture	Herbaceous vegetation grazed by cattle or eventually mowed.
Pond	Standing waterbodies, excluding cattle tanks.
Pond border	Herbaceous vegetation around the pond banks up to 30 m. It may also include small shrubs. The pond border was fenced in 3 out of the 5 ponds studied.
Park / Garden	Areas covered with herbaceous vegetation and sparse trees ( <i>e.g. public parks, large gardens, orchards</i> ).
Residential areas	Individual houses with terraces, small gardens, trees and edges (total individual surface < 2 500 m <sup>2</sup> ).
Trees	Forests, groves and large hedgerows which length > 50 m and width > 10 m in aerial view.

Table 4.S2: Details and source of the CMR data presented in Fig. 4.6.

Species name	Sampling year	Reference
<i>Sympetrum sanguineum</i>	1997	Conrad, 1999
<i>Sympetrum depressiusculum</i>	2012	Dolný et al., 2014
<i>Somatochlora alpestris</i> (1)	1998	Knaus & Wildermuth, 2002
<i>Somatochlora alpestris</i> (2)	2000	Knaus & Wildermuth, 2002
<i>Libellula depressa</i>	2001	Angelibert & Giani, 2003
<i>Urothemis edwardsii</i> (1)	2016 (Lac Bleu)	Khelifa et al., 2016
<i>Urothemis edwardsii</i> (2)	2016 (Lac Noir)	Khelifa et al., 2016
<i>Anax imperator</i>	2017-2018	Minot et al. (present data)



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## **Chapter 5**

### **Diversity and genetic structure of *Anax imperator* populations at the European scale**

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#### **Abstract**

Anthropogenic activities cause the loss and fragmentation of natural habitats. These changes in landscape characteristics can have a strong effect on genetic structure and maintenance of populations by increasing their isolation. Pond ecosystems are scattered waterbodies in the landscape connected in a network by dispersal events of freshwater organisms. Understanding how gene flow is distributed across pond networks and identifying potential local genetic differentiations is essential to prevent risks associated with environmental perturbations. This study aimed to investigate the diversity and genetic structure of *Anax imperator* populations at both the regional and European scale using 7 microsatellites markers. Seven populations of *A. imperator* were sampled in Normandy and 4 populations were sampled in other European countries (*i.e.* Italy, Czech Republic, Swiss and United Kingdom). Normandy populations presented a low genetic differentiation indicating a high gene flow and confirming the high dispersal rate of this species between regional ponds. No pattern of isolation by distance was found at the European scale and populations of continental Europe presented a low genetic structure. Only the U.K. population presented a significant genetic differentiation from other European populations. This result suggests that the English Channel may act as a barrier to gene flow of the large dragonfly *A. imperator*. However, Bayesian analysis showed that some dispersal events could occur from the U.K. to Normandy probably helped by the wind currents of the Gulf stream.

**Key words:** Dispersal barriers, Genetic differentiation, Odonates, Pond network, Population structure

## 1. Introduction

The spatial structure of populations is generally conditioned by intrinsic life traits (*e.g.* dispersal capacities), distances between sites and environmental factors such as climate gradients or physical barriers (Thomas & Kunin 1999; Sexton et al. 2014; Norman 2017). Anthropogenic activities change the landscape characteristics leading to the loss and fragmentation of natural habitats (Maxwell et al. 2016). As a consequence, many wildlife populations have to live in increasingly isolated habitat patches and suffer a loss of genetic diversity due to inbreeding (Frankham et al. 2010; Dennis et al. 2013). Studies on the genetic diversity are crucial to drive species conservation measures because a low genetic diversity increases the risk of population extinction due to environmental perturbations and demographic stochasticity (Allendorf et al. 2013). The local genetic diversity can vary independently from the geographical distribution of the considered species. Some wide-ranging species with a priori high dispersal capacities can have a different genetic diversity at smaller scale (Bergamaschi et al. 2015), while other ones with low dispersal ability can have low genetic differentiation at larger spatial scales (De Meester et al. 2002, Habel et al. 2010). In rare species, the genetic diversity can also be constrained by specific habitat requirement that induce an isolation of populations (Heinken & Weber 2013; Kohli et al. 2018). This genetic diversity is alimanted by gene flow that depends on the rate of dispersal events between populations. High gene flow will prevent local genetic differentiation while reduced gene flow due to rare dispersal events will lead to genetic structuration (Ronce 2007).

Most dispersal events cover only short distances (Short Distance Dispersal; SDD) and stay within the boundaries of a defined geographic or population limits (Jordano 2017). However, these SSD events may also allow to connect distant populations by 'step by step' dispersal process (Le Corre & Kremer 1998; Coughlan et al. 2017). On the contrary, Long Distance Dispersal (LDD) movements are rare and difficult to detect (Nathan 2006; Fonte et al. 2019), except in migratory species (Newton 2008; Brink et al. 2018). LDD often involves natural forces like wind and marine currents (Bowen & Karl 2007; Haest et al. 2019) or relies on other organisms with higher dispersal abilities (Green & Figuerola 2005; Coughlan et al. 2017). In most species, dispersal events are scarce, but have major consequences on population dynamics. They can either enable the colonisation of new patches to expand the species occupancy on the territory or be involved in genetic mixing with other populations (Jordano 2017). Both are crucial for population maintenance, because they enable to spread the risk of extinction on several sites and to maintain genetic diversity (Baguette et al. 2013) that allows to increase the resilience of populations to environmental changes (*e.g.* climate

change, exotic species, habitat fragmentation; Stockwell et al. 2003; Kelly & Phillips 2016). Measuring the amount of dispersal can be difficult on the field, but genetic techniques can provide accurate estimates on gene flow between populations and therefore indirect measurements of dispersal (Ross 2001; Leggett 2011).

Ponds are small waterbodies giving rise to an increasing conservation interest because of their non-negligible biodiversity of aquatic plants, macro-invertebrates and amphibians (Biggs et al. 2017; Hill et al. 2017). Although ponds are scattered elements in the landscape, they are increasingly considered as working in networks. Interestingly, stepping stone models (Kimura 1953) can be applied on these ecosystems to investigate the genetic structure of pond populations (Watts et al. 2015; Youngquist et al. 2017). The persistence of populations is constrained by the availability of suitable habitats, the distance between ponds and the dispersal capacities of the considered species (Thornhill et al. 2018). Pond populations are also threatened by perturbations like water pollution or summer droughts (Oertli et al. 2005; Anderson et al. 2015; Biggs et al. 2017). Determining how gene flow is distributed across the pond networks and identifying potential local genetic differentiations is essential to understanding population structure and assessing risks associated with environmental perturbations and fragmentation.

Odonates are insects with an aquatic larval development and a terrestrial adult stage (Suhling et al. 2015). While some rare movements were reported at the larval stage, the vast majority of dispersal events is performed by flying adults (Bilton et al. 2001). Dispersal distances are difficult to quantify, but may vary a lot depending on the dispersal abilities of the species. In many species, most individuals stay on the same pond during their whole lifetime (Conrad et al. 1999; Rouquette & Thompson 2007; Le Gall et al. 2017), whereas other species like *Pantala flavescens* can undertake recurrent migration flights across the oceans (Troast et al. 2006). Whether for supporting active migrations of large species (e.g. May 2013; Knight et al. 2020) or blowing small passive species on long distances (Suhling et al. 2009), the wind plays a major role in the dispersal of odonates. The degree of genetic differentiation between odonate populations is very variable among species and do not only depend on their body size, but also ecological niche. For instance, some specialists like *Coenagrion mercuriale* (Lorenzo-Carballea et al. 2015) presented a marked population differentiation for a distance of 24 km, whereas very weak genetic structure was found at European scale for the generalist species *Ishnura elegans* (Wellenteuther et al. 2011). Several landscape features might also act as a physical barrier to dispersal, limiting the gene flow between populations. For instance, small

hills or patches of trees and shrubs prevented the dispersal movements of *C. mercuriale* (Purse et al. 2003; Watts et al. 2006)

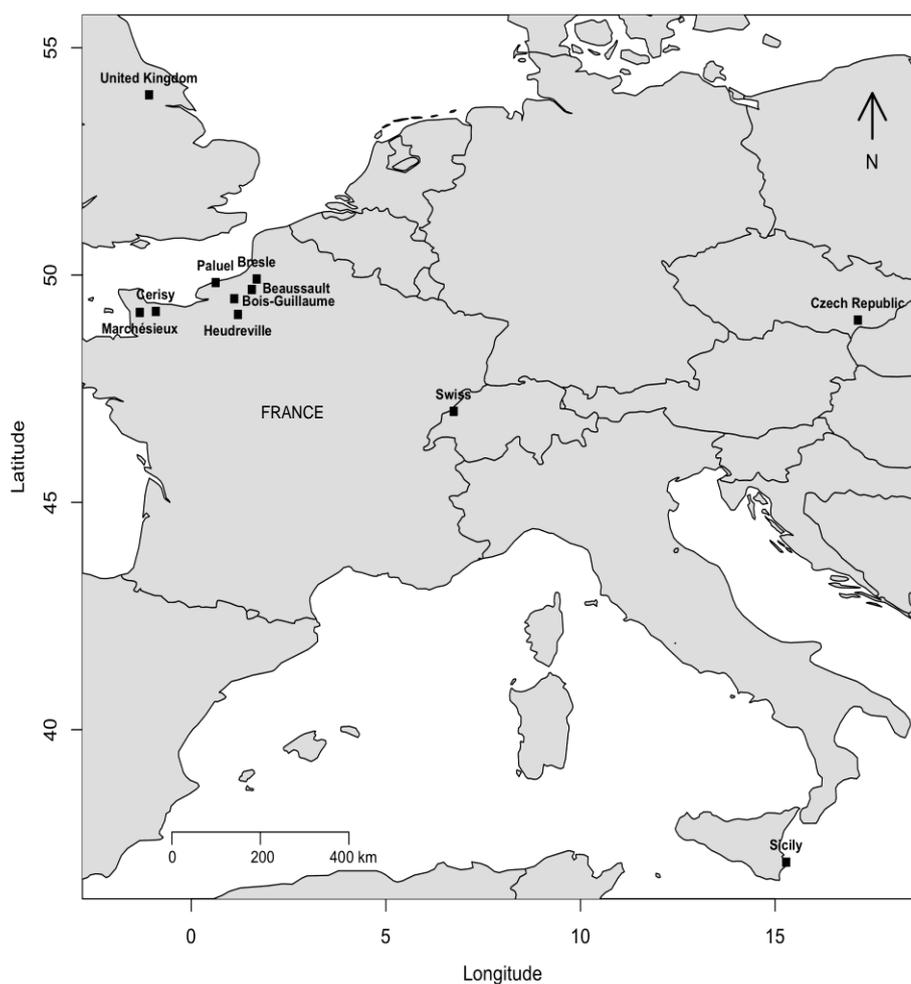
*Anax imperator* is the largest dragonfly species in Western Europe (Dijkstra 2015). Although no migration has been reported for this species (Corbet, 1999), its distribution ranges from South Africa to the United Kingdom (Tarboton & Tarboton 2015). Mature adults are very mobile and breed on large sun-exposed ponds with aquatic vegetation (Corbet 1957). They fly very fast and often stay away from the bank of the ponds, a behaviour that makes them hard to catch. The exuviae measures ca. 5 cm (Minot et al. 2019) and can be found, sometimes in high densities, on the bank vegetation of ponds (Corbet 1957).

This study focused on 7 pond populations of *Anax imperator* from the Normandy Region (France), and 4 pond populations from other European countries (*i.e.* Italy, Czech Republic, Swiss and United Kingdom). Genetic diversity and gene flow were investigated between sites at the Normandy scale (*i.e.* regional scale) and at the European scale. Samples consisted either in leg of adults, leg of larvae, fresh exuviae (*i.e.* collected within the 24h after ecdysis) or old exuviae. Therefore, the DNA exploitability between fresh and old exuviae were compared. Since *A. imperator* is a large dragonfly with high dispersal abilities between its breeding sites, only a weak genetic differentiation was expected between populations at a regional scale. We also hypothesized that geographical barriers, such as large seas or mountain chains, would limit gene flow between populations at the European scale.

## 2. Material and methods

### 2.1. Study populations and sample collection

Samples were collected in 11 localities in Europe distributed among 5 countries (*i.e.* United Kingdom, France, Swiss, Czech Republic and Italy (Sicily)); Fig. 5.1 & Table 5.1). A total of 251 individuals were collected for their DNA. Depending on localities, different types of samples were collected: a mid-leg tibia of adults, a mid-leg tibia of larvae, fresh or old exuviae (Table 5.1). Some exuviae used for the analyses come from individuals that were reared at the University of Rouen (Normandy, France). They were collected within the 24 following ecdysis and were therefore qualified of “fresh”. Other exuviae sampled on the field were qualified of “old” because the date of ecdysis was unknown and this exoskeleton can persist over several weeks in the vegetation (Lubertazzi & Ginsberg 2009; Roland 2010). After collection, all samples were stored in absolute alcohol until DNA extraction.



**Fig. 5.1.** Map showing the 11 *Anax imperator* populations sampled in Europe. Characteristics of each population are referenced in Table 5.1.

**Table 5.1.** Characteristics of the 11 populations of *Anax imperator* in Europe and the number of collected samples according to their source of DNA.

Country	Pop	Site name	Coordinates (WGS84)	Source of DNA			
				Adult legs	Larval legs	Fresh exuviae	Old exuviae
Sicily	1	-	37.086 N, 15.286 E	6			
Swiss	2	-	47.002 N, 6.741 E	16			
Czech Republic	3	-	49.010 N, 17.128 E		16		12
France	4	Beaussault	49.682 N, 1.555 E		33		
France	5	Cerisy	49.199 N, -0.912 E		19		
France	6	Heudreville	49.133 N, 1.198 E			36	4
France	7	Bois- Guillaume	49.480 N, 1.102 E			39	
France	8	Marchésieux	49.178 N, -1.324 E		14		
France	9	Paluel	49.835 N, 0.624 E			15	
France	10	Bresle	49.914 N, 1.679 E				10
United Kingdom	11	-	53.964 N, -1.086 E				31
Sum (n = 251)				22	82	90	57

## 2.2. DNA extraction and microsatellite genotyping

Collected legs were manually reduced in smaller fragments using scissors. Then, DNA extraction was performed using QIAamp Micro kits (QIAGEN France) and following the protocol provided. Collected exuviae were dried on a glass surface during the night before extraction to allow alcohol evaporation. Then, the head was removed, and the exuviae was cut with scissors to keep only the thorax, legs and the white tracheal lining from the abdomen. The material was placed in a 5 ml Eppendorf tube with 3 steel beads and homogeneously grinded with a MM400 mixer mill (Retsch) during 3 minutes according to the protocol proposed by Keller et al. (2009). Finally, DNA extraction was performed using DNeasy Blood & Tissue Kits (QIAGEN France) and following the protocol provided except the following changes. Quantity of proteinase K was 25  $\mu$ L, quantities of buffer AL and ethanol were 250  $\mu$ L after incubation and as suggested by Keller et al. (2009), the elution step was performed twice with 50  $\mu$ L AE buffer. Individuals were genotyped using 12 microsatellite loci previously developed for *A. imperator* (Hadrys et al. 2007). Among these loci, 2 were derived from the sister species *A. parthenope* and showed successful amplification with *A. imperator*. Primers 5'-labelled with 3 fluorescent dyes (*i.e.* FAM, VIC and PET; Life Technologies SAS) were used to amplification reaction in four separate PCR multiplexes in a thermocycler (Mastercycler nexus gradient Eppendorf). Polymerase Chain Reactions (PCRs) were performed in total volumes of 12.5  $\mu$ L containing 6.25  $\mu$ L Qiagen Multiplex PCR Master Mix (QIAGEN France), 4  $\mu$ L RNase-free water, 1.25

$\mu\text{L}$  of one of the four multiplexed primer combinations (concentration of each primer:  $2 \mu\text{mol}/\mu\text{L}$ ) and  $1 \mu\text{L}$  of DNA ( $10 \text{ ng}/\mu\text{L}$ ). PCR were performed using the following thermocycler program (QIAGEN): an initial denaturation step at  $95^\circ\text{C}$  for 15 min, followed by 35 cycles of denaturation at  $93^\circ\text{C}$  for 30 s, annealing for 90 s at  $52^\circ\text{C}$  or  $57^\circ\text{C}$  depending on the multiplexes used and an elongation at  $72^\circ\text{C}$  for 60 s, and a final extension at  $65^\circ\text{C}$  for 30 min. Finally,  $1 \mu\text{L}$  of each PCR product was added to a solution of  $8.8 \mu\text{L}$  of formamide (Applied Biosystems) and  $0.2 \mu\text{L}$  of GeneScan 600 LIZ size standard (Applied Biosystems). Fragments were sized on an ABI Prism and analysed by capillary electrophoresis using the 3500 Genetic Analyzer (Applied Biosystems). Genetic data were analysed with GeneMapper 4.1 software (Applied Biosystems).

### 2.3. Genetic diversity

All analyses were performed using the R software (R Core Team 2019). Means are given  $\pm$  SD.

The presence of null alleles was checked using the R package “PopGenReport” (Adamack & Gruber 2014). Deviation from expected Hardy-Weinberg Equilibrium (HWE) conditions for each locus and each population was tested using the R package “pegas” (Paradis 2010) and an exact test based on 10,000 Monte Carlo permutations of alleles. Linkage Disequilibrium (LD) between all locus-pair combinations was tested using the R package “genepop” (version 1.1.7: Raymond & Rousset 1995; Rousset 2008). Markov chain parameters were 1,000 dememorization, 100 batches and 1,000 iterations per batch for each test. P-values in the detection of HWE and LD were corrected with a False Discovery Rate (FDR) procedure using Benjamini-Hochberg-Yekutieli method (Benjamini & Hochberg 1995; Benjamini & Yekutieli 2001).

Observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), numbers of alleles ( $N_a$ ), allelic richness ( $A_R$ ) and inbreeding coefficients ( $F_{IS}$ ) for each population were calculated using the R package “diveRsity” (version 1.9.90; Keenan et al. 2013). The  $A_R$  was calculated using the rarefaction method to correct for variation in sample size (Kalinowski 2004) and to avoid having to exclude a population from analyses. 95% Confidence Intervals (CI) for  $F_{IS}$  estimates were calculated using 10,000 bootstrap iterations.

The global measures of  $F_{IS}$  and  $F_{ST}$ , as well as pairwise  $F_{ST}$ -values between all populations, were calculated using the DiveRsity package.  $F_{ST}$  is considered as an effective measure for population genetic differentiation when using relatively small data sets with fewer than 20 loci (Gaggiotti et al.

1999, Whitlock & McCauley 1999). All these  $F$ -statistics used the bias-corrected formulation of Weir and Cockerham (1984). Pairwise values of the bias-corrected Jost's  $D_{\text{est}}$  between all populations were also calculated using the *diveRsity* package.

Estimate 95% confidence intervals for all measures of differentiation were calculated using 10,000 bootstrap iterations.  $D_{\text{est}}$  is a relative measure of differentiation, which range from 0 (*i.e.* no differentiation) to one (*i.e.* complete differentiation), and simulations have shown that it is an unbiased estimator of differentiation, and outperforms  $F_{\text{ST}}$ , over a range of sample sizes and for markers with different numbers of alleles (including highly variable microsatellite loci; Gerlach et al. 2010). Correlation between pairwise  $F_{\text{ST}}$  and  $D_{\text{est}}$ -values was tested using a Mantel test with 10,000 permutations and “*ade4*” package (Dray et al. 2007).

#### 2.4. Population genetic analyses and geographic structure

We used the *pegas* package (Paradis 2010) to perform an Analysis of MOlecular VAriance (AMOVA; Excoffier et al. 1992) based on Euclidian distances among individuals for all microsatellite loci. The AMOVA was conducted to partition total genetic variation across three hierarchical levels: among countries (*i.e.* UK, France, Swiss, Czech Republic and Sicily), among populations within countries and within populations. The statistical significance of the fixation indexes  $\Phi$  was calculated using 10,000 permutations of data.

Genetic Isolation-by-distance (IBD), which is defined as a decrease in a genetic similarity among populations as the geographical distance between them increases, was tested using a Mantel test with 10,000 permutations and “*ade4*” package. A matrix of genetic differentiation using  $F_{\text{ST}}/(1-F_{\text{ST}})$  according to Rousset (1997) and a matrix of Euclidean distances between the populations were performed between *A. imperator* populations.

In order to investigate the genetic structure of the 11 sampling populations of *A. imperator*, a model-based clustering was performed using the *STRUCTURE* 2.3.4 program (Pritchard et al. 2000). It uses a Bayesian Markov chain Monte Carlo (MCMC) method to identify genetic clusters ( $K$ ) and assign individuals to these clusters. Each cluster is characterised by a set of allele frequencies at each locus. Individuals are assigned to these clusters based on the likelihood of their multilocus genotypes to belong to these genetic clusters and this, by minimising deviations from Hardy-Weinberg

equilibrium (HWE) and linkage disequilibrium (LD; Pritchard et al. 2000). We performed runs for a number of clusters (K) ranging from 2 to 8 and with a number of 20 independent runs for each K. *Anax imperator* was expected to have high dispersal abilities leading to frequent exchanges of individuals between populations. Therefore, an admixture model with correlated allele frequencies was considered. The LOCPRIOR parameter was not considered, *i.e.* the geographic location of the individuals was not considered as an additional information. For each model, a burn-in period of 100 000 followed by 1 000 000 iterations was used to ensure convergence of the MCMC. The optimum number of clusters was identified using the log-likelihood ( $\ln P(K)$ ) and the estimated  $\Delta K$  for each K following Evanno et al. (2005). CLUMPP (Jakobsson & Rosenberg 2007) was used to aggregate all STRUCTURE runs for the optimum identified value of K. STRUCTURE models, identification of K following the Evanno's method, CLUMP analyses and visualisation of the individual Bayesian assignment probability for the optimum value of K were performed using the R package STRATAG (Archer et al. 2017).

Since French populations sampled were geographically close, we also investigated genetic structure in individuals using a Discriminant Analysis of Principle Components (DAPC) performed with the R package ADEGENET 2.1.3 (Jombart 2008).

Spatial genetic structure was also investigated using a spatial model in the R package GENELAND 4.9.2 (Guillot et al. 2005). Like STRUCTURE, GENELAND provides tools to identify clusters of individuals using Bayesian MCMC inferences with genetic data by maximising Hardy-Weinberg Equilibrium and minimising Linkage Disequilibrium, but geographical coordinates of individuals are also considered to inform prior distribution. This spatial clustering method allows to infer the borders between inferred clusters and is a powerful method for detecting linear barriers to gene flow between populations (Blair et al. 2012). GENELAND analysis was performed using four independent runs and for each run, a number of clusters K ranging from  $K_{min} = 1$  to  $K_{max} = 8$ , 1 000 000 MCMC iterations, a burn-in period of 1000 and a thinning value of 100. We considered a correlated allele frequency model that took into account the potential presence of null alleles. The best run was selected according to the highest average posterior probability given by GENELAND.

## 2.5. Migration rates between studied populations

In order to estimate recent migration rates between populations (*i.e.* over the last several generations), analyses using MCMC were done in BAYESASS 3.0.4 software (Wilson & Rennala 2003). The model was first run considering default values of the mixing parameters for migration rates (*i.e.* 0.1), allele frequencies (*i.e.* 0.1) and inbreeding coefficients (*i.e.* 0.1). The acceptance rates given by BAYESASS of each of these 3 mixing parameters must be comprised optimally between 20% and 60%. Since the acceptance rates were first higher to 60%, the model used ran with higher values of the mixing parameters (*i.e.* 0.7 for migration rate, 0.65 for allele frequencies and 0.8 for inbreeding coefficients) and a burn-in of  $1 \times 10^6$  for  $1 \times 10^7$  iterations.

## 3. Results

### 3.1. Genetic diversity

Two of the 12 considered microsatellites (*i.e.* AiKo4 and AiGo3) were not retained because the first was monomorphic and the other was not successfully amplified. Loci AiJo4, AiLo4 and AiMo4 had high frequencies of null alleles (0.25, 0.25 and 0.23 respectively) and were also not retained. Then, individuals with more than 3 loci with missing values were removed from the data. No sample issued from adult or larva legs was excluded. Ten samples issued from fresh exuviae were excluded (*i.e.* 11.1% of the total number of fresh exuviae) and 18 samples issued from old exuviae were excluded (*i.e.* 31.6% of the total number of old exuviae). Finally, 223 individuals were considered for further analyses on 7 markers (Table 5.2) and the total remaining missing values represented 6.6% of the loci.

Globally, populations showed substantial genetic variations. Estimates of observed and expected heterozygosity were close and ranged from 0.33 to 0.75 and from 0.61 to 0.72 respectively (Table 5.2). Only the population of United Kingdom presented an observed heterozygosity (0.33) smaller than the expected heterozygosity (0.63). The total number of alleles over all loci ranged from 29 alleles in the population of Sicily to 60 alleles in the population 5 of France (Table 5.2). A significant positive correlation ( $r = 0.84$ ) was observed between the number of sampled individuals and the total number of alleles over all loci (Pearson correlation test:  $t = 4.59$ ,  $df = 9$ ,  $P = 0.0013$ ). Estimates of allelic richness per locus were similar between populations (Table 5.2).

Most markers met HWE conditions in each population except in the two populations showing departure from HWE conditions (*i.e.* U.K. and Heudreville,  $P < 0.05$ ; Table 5.2). Because these departures of markers from HWE were not systematic in all populations, all markers were retained for further analyses. All other populations met HWE conditions ( $P > 0.05$ ).  $F_{IS}$  values showed significant deviation from zero in the two populations that did not meet HWE, indicating homozygosity excess in these two populations, and especially in the UK population (Table 5.2). Linkage disequilibrium (LD) tests for each pair of loci over all populations indicated no evidence for significant disequilibrium (all  $P > 0.05$ ).

**Table 5.2.** Genetic diversity measures (mean  $\pm$  SE) in the 11 sampled populations of *Anax imperator* from Europe.  $n$  = number of sampled individuals,  $H_o$  = observed heterozygosity,  $H_e$  = expected heterozygosity,  $N_a$  = number of alleles,  $A_R$  = allelic richness,  $F_{IS}$  = inbreeding coefficient. Bolded  $H_o$  indicate populations presenting a significant departure from HWE condition. Bolded  $F_{IS}$  indicate a bootstrapped 95% confidence interval that does not overlap zero.

Country	Pop	n	$H_o$	$H_e$	$N_a$	$A_R$	$F_{IS}$
Sicily	1	6	0.71 $\pm$ 0.07	0.62 $\pm$ 0.05	29	4.14 $\pm$ 0.46	-0.17
Swiss	2	16	0.65 $\pm$ 0.06	0.61 $\pm$ 0.06	42	4.02 $\pm$ 0.44	-0.06
Czech Republic	3	21	0.61 $\pm$ 0.07	0.65 $\pm$ 0.06	47	4.26 $\pm$ 0.57	0.06
France	4	33	0.61 $\pm$ 0.07	0.65 $\pm$ 0.06	56	4.39 $\pm$ 0.52	0.06
France	5	19	0.59 $\pm$ 0.08	0.68 $\pm$ 0.05	46	4.40 $\pm$ 0.54	0.15
France	6	32	<b>0.47 <math>\pm</math> 0.06</b>	0.67 $\pm$ 0.06	60	4.75 $\pm$ 0.54	<b>0.30</b>
France	7	34	0.59 $\pm$ 0.06	0.69 $\pm$ 0.05	54	4.63 $\pm$ 0.45	<b>0.16</b>
France	8	14	0.75 $\pm$ 0.07	0.72 $\pm$ 0.04	46	4.76 $\pm$ 0.47	-0.03
France	9	14	0.57 $\pm$ 0.06	0.66 $\pm$ 0.05	45	4.54 $\pm$ 0.38	0.15
France	10	9	0.43 $\pm$ 0.08	0.61 $\pm$ 0.06	31	4.05 $\pm$ 0.53	0.25
United Kingdom	11	25	<b>0.33 <math>\pm</math> 0.11</b>	0.63 $\pm$ 0.05	37	3.96 $\pm$ 0.35	<b>0.51</b>

### 3.2. Population genetic differentiation

Global  $F_{IS}$  (0.1615, 95% CI = 0.1233 – 0.2000) and  $F_{ST}$  (0.0224, 95% CI = 0.0101 – 0.0366) were greater than zero. Pairwise  $F_{ST}$ -values ranged between -0.0151 and 0.1297. Global  $D_{est}$  was greater than zero (0.0410, 95% CI = 0.0038 – 0.0850) and pairwise  $D_{est}$ -values ranged between -0.0006 and 0.1462. Pairwise  $F_{ST}$  and  $D_{est}$ -values were highly correlated (Mantel test:  $r_2 = 0.97$ ,  $P < 0.001$ ; Table 5.3).

Moderate genetic differentiation (*i.e.* 95% bootstrapped CI) was particularly found between UK and all other populations (all  $F_{ST} > 0.081$ ). Moderate differentiation was also found between the

Swiss population and a French population (*i.e.* Marchésieux,  $F_{ST} = 0.046$ ).  $D_{est}$ -values showed similar pattern to the pairwise  $F_{ST}$ -values but with slightly higher values (Table 5.3).

**Table 5.3.**  $D_{est}$ -values (above diagonal) and  $F_{ST}$ -values (below diagonal) between all populations. Bolded values indicate a bootstrapped 95% confidence interval that does not overlap zero. The mean  $D_{est}$ -value is  $0.0407 \pm 0.0060$  and the mean  $F_{ST}$ -value is  $0.0306 \pm 0.0051$ .

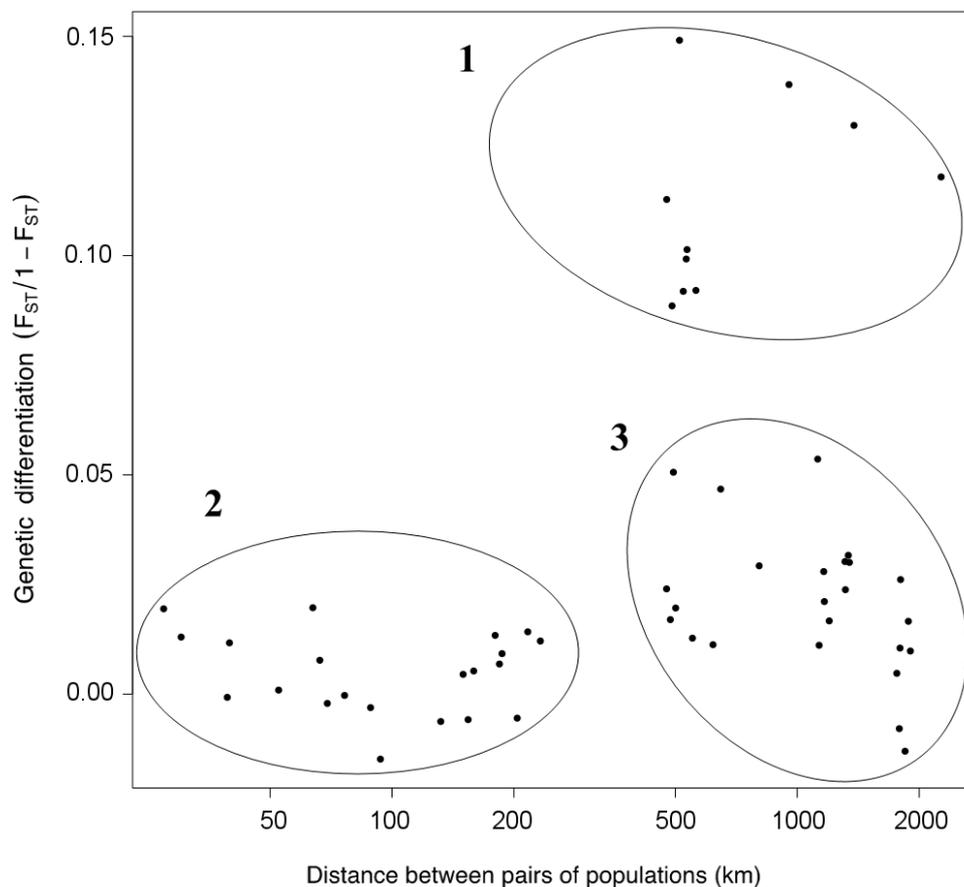
	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11
Pop1	-	0.0293	0.0307	-0.0079	0.0163	0.0047	0.0104	0.0097	-0.0132	0.0254	<b>0.1055</b>
Pop2	0.0147	-	0.0128	0.0167	0.0111	0.0234	0.0192	<b>0.0446</b>	0.0126	0.0481	<b>0.1220</b>
Pop3	0.0252	0.0284	-	0.0110	0.0232	0.0272	0.0206	0.0291	0.0164	0.0509	<b>0.1148</b>
Pop4	0	0.0223	0.0109	-	0.0037	0.0115	0.0143	0.0036	-0.0021	0.0191	<b>0.1297</b>
Pop5	0.0081	0.0093	0.0318	0.0091	-	-0.0059	0.0045	0.0049	-0.0063	-0.0055	<b>0.0903</b>
Pop6	0.0029	0.0227	0.0346	0.0077	-0.0001	-	-0.0007	0.0019	-0.0031	-0.0151	<b>0.0843</b>
Pop7	0.0086	0.0246	0.0258	0.0115	0.0008	0.0003	-	0.0183	0.0009	0.0193	<b>0.0841</b>
Pop8	0.0014	0.0789	0.0358	0.0140	0.0128	0.0068	0.0132	-	0.0052	0.0119	<b>0.0920</b>
Pop9	0	0.0141	0.0282	0.0004	-0.0006	0.0001	0.0034	0.0006	-	0.0043	<b>0.1014</b>
Pop10	0.0144	0.0466	0.0525	0.0295	0.0007	0	0.0254	0.0023	-0.0003	-	0.0813
Pop11	<b>0.1200</b>	<b>0.1462</b>	<b>0.0993</b>	<b>0.1423</b>	<b>0.1284</b>	<b>0.0954</b>	<b>0.0997</b>	<b>0.1087</b>	<b>0.1431</b>	0.0612	-

The AMOVA analysis showed that most molecular genetic variation occurred within populations (92.73%; Table 5.4). Genetic variation was also significant among countries (6.37%,  $P = 0.02$ ). Variation among populations within countries concerned only French populations, since there was only one population for the other countries. In France, no significant genetic variation was found among populations (0.90%,  $P = 0.09$ ).

**Table 5.4.** Results of the AMOVA performed for the 11 population of *Anax imperator* within four European countries.

Source of variation	df	Sum of squares	Variance components	Percentage of variance	$\Phi$ -statistics	P-value
Among countries	4	138.79	0.78	6.37	$\Phi_{CT} = 0.06$	0.02
Among populations within countries	6	82.21	0.11	0.90	$\Phi_{SC} = 0.01$	0.09
Within populations	212	2405.45	11.35	92.73	-	-
Total	222	2626.45	12.24	100.00	-	-

No evidence for isolation by distance was found among populations at the European scale, since the correlation between genetic and geographic distance matrices was not significant (Mantel test:  $r = 0.18$ ,  $P = 0.19$ ; Fig. 5.2). However, when considering UK and French populations only, an isolation by distance tends to occur between the two countries (Mantel test:  $r = 0.90$ ,  $P = 0.08$ ; Fig. 5.2).

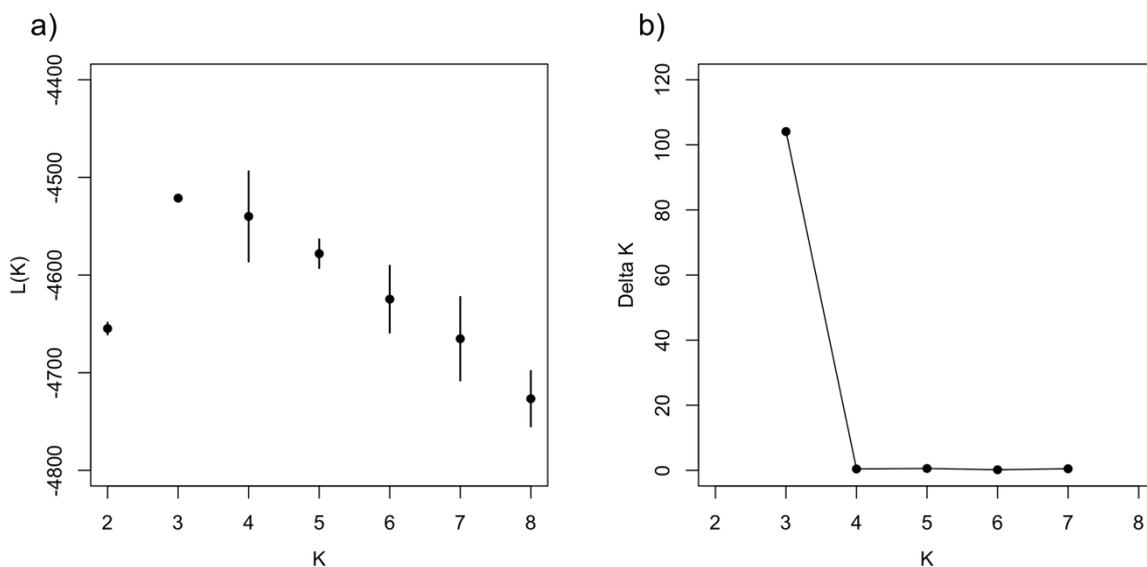


**Fig. 5.2.** Relationship between pairwise population differentiation ( $F_{ST}/1 - F_{ST}$ ) and the distance separating populations ( $\ln$  km). Ellipse 1 represents pairwise differentiations between UK and all other populations. Ellipse 2 represents pairwise differentiations between Normandy populations. Ellipse 3 represents pairwise differentiations between Normandy populations and the three Eastern populations (i.e. Swiss, Czech Republic and Sicily) and the three Eastern populations between them.

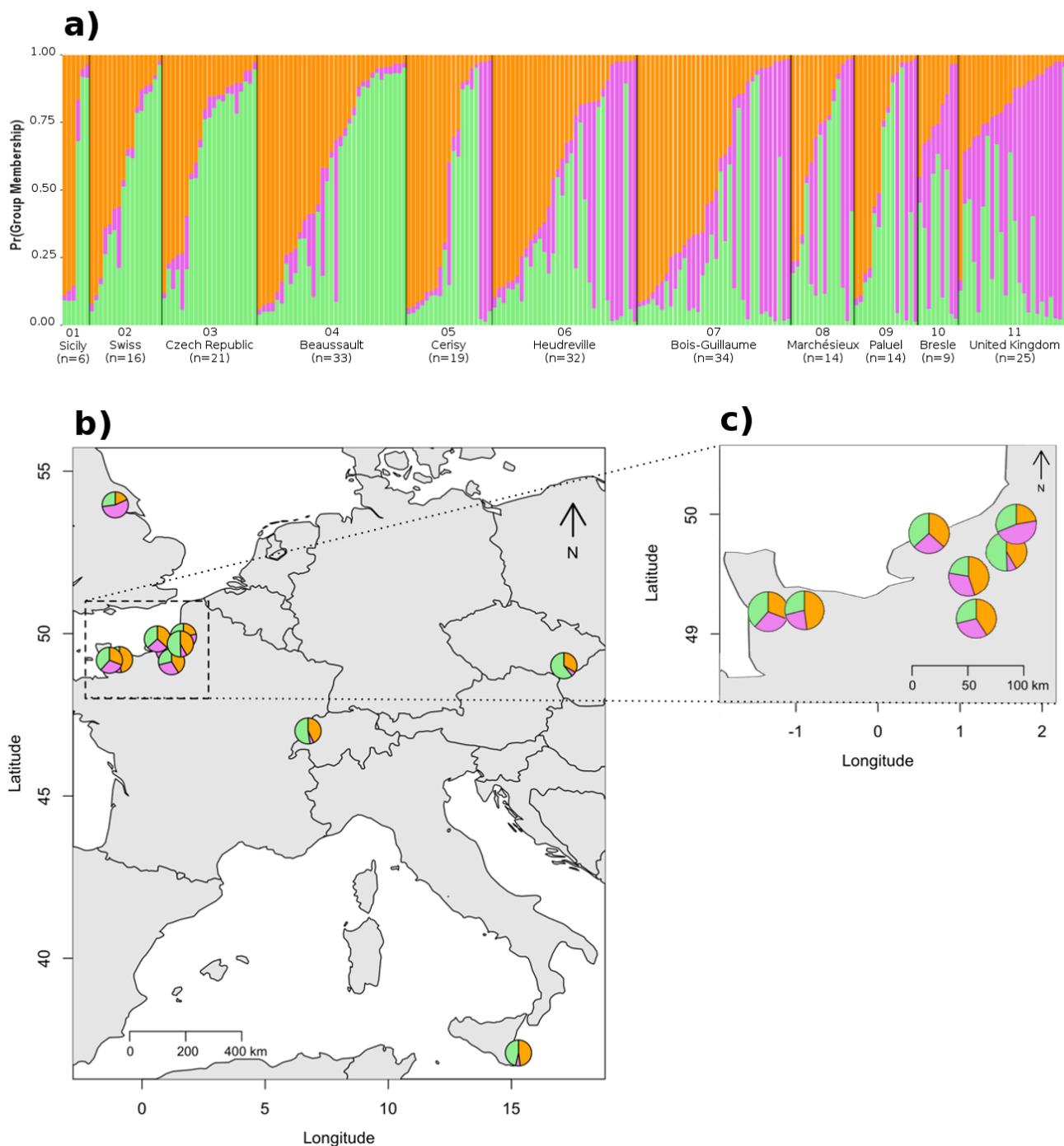
### 3.3. Spatial genetic structure

STRUCTURE analyses allowed to identified 3 genetic clusters since values of  $\ln P(K)$  and  $\Delta K$  showed a peak at  $K = 3$  (Fig. 5.3a & 3b). The results show a first group containing populations from Czech Republic, Swiss and Sicily (Figs. 4a & 4b). A second group contained the UK population (Fig. 5.4a & b). Finally, all French populations seemed to be a mix of the two other groups (Fig. 5.4a & c).

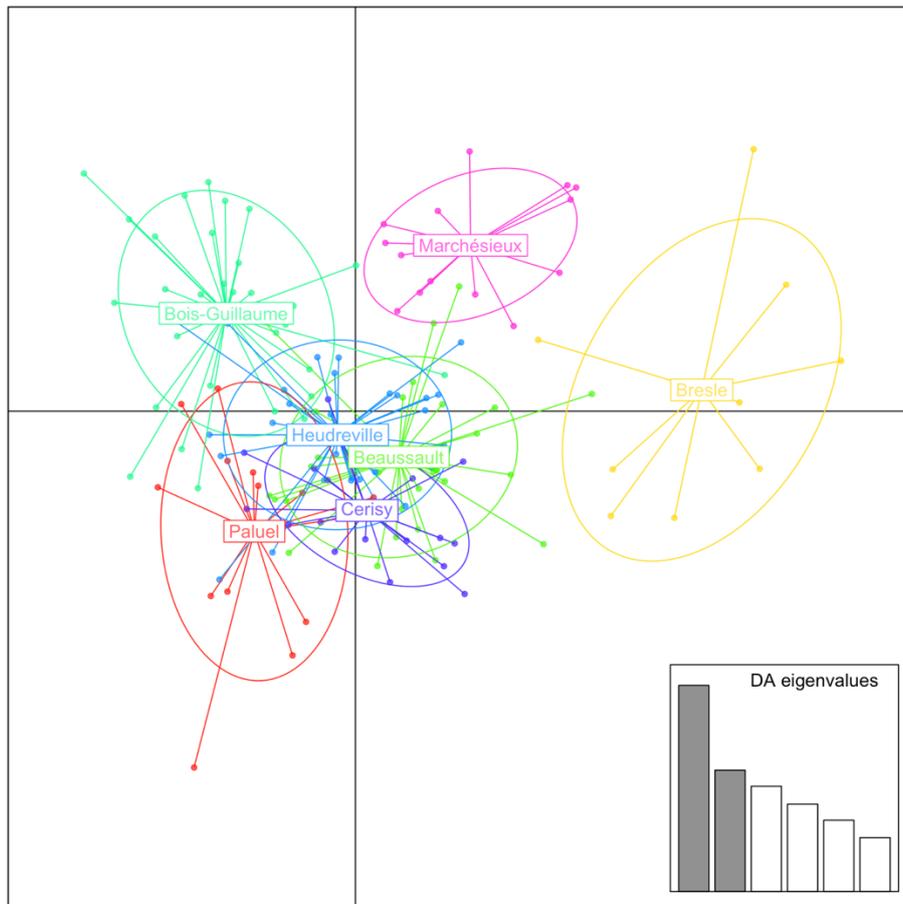
Discriminant Analysis of Principle Components (DAPC) on French populations was performed retaining 70 Principal Components (PC) and 2 discriminant functions. It suggested 3 subclusters: one with the population of Bresle, a second with the population of Marchésieux and a third with the five other Normandy populations in which the population of Bois-Guillaume was slightly detached from the four other populations (Fig. 5.5).



**Fig. 5.3.** Estimation of number of clusters ( $K$ ) with STRUCTURE using a) mean of estimated  $\ln P(K)$  for each  $K$ -value and b) Delta  $K$  for each  $K$ -value (Evanno's method).

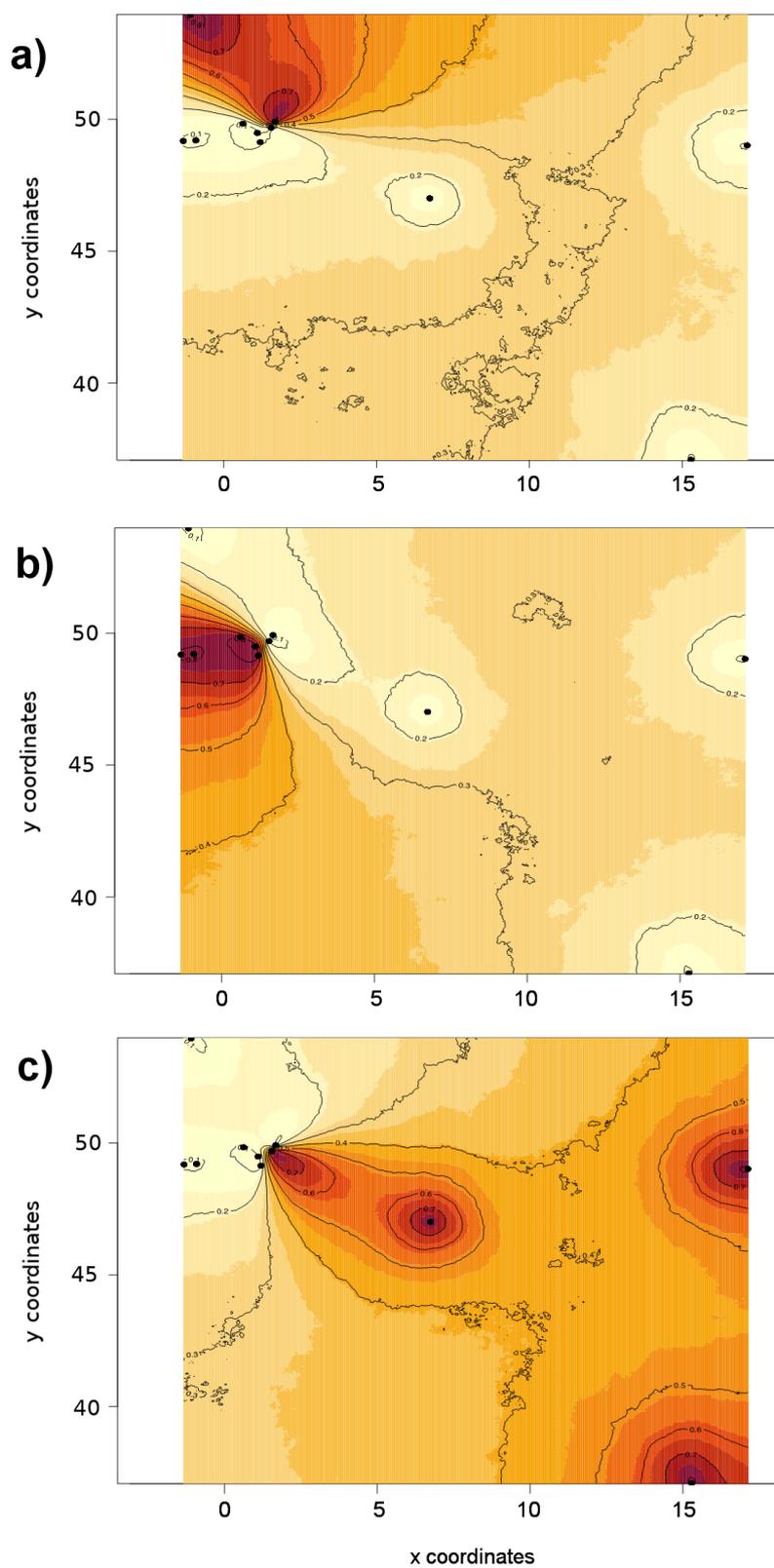


**Fig. 5.4.** Results of individual assignments to each cluster by STRUCTURE. a) Probabilities of individual membership in the 11 sampled populations, with bars representing individuals and colours the probability to belong to the three genetic clusters identified with STRUCTURE. b) Mean membership in the 11 sampled populations in Europe to each of the three clusters. French sampled populations are delimited in a dotted rectangle. c) Enlarged view of the French sampled populations.



**Fig. 5.5.** Discriminant Analysis of Principle Components results of *Anax imperator* individuals of sampled French populations.

All independent runs performed in the spatial model given by GENELAND corroborated STRUCTURE results and identified 3 genetic clusters. The run with higher average log posterior probability was retained. MCMC converges within the 100 000 iterations. UK and Bresle populations were assigned to one cluster with a probability of at least 0.7 (Fig. 5.6a). Most other Normandy populations excepted Beaussault were assigned to a second cluster with a probability of at least 0.7 (Fig. 5.6b). Normandy population of Beaussault and populations of Swiss, Czech Republic and Sicily were assigned to a third cluster with a probability of at least 0.7 (Fig. 5.6c).



**Fig. 5.6.** Results of spatial Geneland analysis on the 11 populations of *Anax imperator*. Each figure corresponds to a cluster identified by Geneland. Black dots indicate the position of the populations (see Fig. 5.1). Black lines indicated the posterior probabilities of membership in the three clusters, with darker colours (red) indicating highest posterior probabilities of belonging to the cluster.

### 3.4. Recent migration rates among populations

Bayesian analyses showed clearly several directional gene flow between studied populations. UK population was rather isolated from the other populations but was a donor site only for the Bresle population in France (Table 5.5). All the 3 countries situated to the East of France (*i.e.* Swiss, Sicily and Czech Republic) showed no migratory exchange between them and were not sources for French populations. French population of Beaussault and especially French population of Cerisy were sources for Swiss, Sicily and Czech Republic populations (Table 5.5). In France especially, Cerisy population was identified as likely one on the main donor for the other four French populations. Heudreville and Beaussault populations seemed particularly implicated in source-sink processes, since they acted both as donor and target populations. French populations of Paluel and Bois-Guillaume were identified as receivers only. The French population of Marchésieux seemed isolated from all other populations since no migratory exchange was identified (Table 5.5).

**Table 5.5.** Bayesian modelling of potential bias in direction of dispersal (gene flow) among the 11 populations studied of *Anax imperator* in Europe. Numbers represent the proportion that disperses between sites (bold indicated self-recruitment). Values < 0.05 (5%) are in grey. Italic indicates pairs of sites with  $\geq 10\%$  exchange. These values represent historical gene flow, and do not provide any information about contemporary levels of dispersal among sites.

Target	Potential donor site										
	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11
Pop1	<b>0.68</b>	0.02	0.02	0.05	0.10	0.02	0.02	0.02	0.02	0.02	0.02
Pop2	0.01	<b>0.68</b>	0.02	0.07	0.12	0.01	0.03	0.01	0.01	0.01	0.01
Pop3	0.01	0.02	<b>0.69</b>	0.04	0.17	0.01	0.02	0.01	0.01	0.01	0.01
Pop4	< 0.01	0.02	0.03	<b>0.69</b>	0.18	0.01	0.01	0.01	< 0.01	< 0.01	< 0.01
Pop5	0.01	0.02	0.03	0.03	<b>0.78</b>	0.02	0.02	0.01	0.01	0.01	0.04
Pop6	< 0.01	0.01	0.01	0.03	0.13	<b>0.72</b>	0.04	< 0.01	< 0.01	< 0.01	0.02
Pop7	< 0.01	0.01	0.02	0.02	0.12	<b>0.06</b>	<b>0.73</b>	< 0.01	< 0.01	< 0.01	0.01
Pop8	0.02	0.02	0.02	0.04	0.10	0.04	0.02	<b>0.69</b>	0.01	0.01	0.03
Pop9	0.01	0.02	0.02	0.05	0.08	0.05	0.02	0.02	<b>0.68</b>	0.01	0.02
Pop10	0.02	0.02	0.02	0.04	0.04	0.03	0.02	0.02	0.02	<b>0.68</b>	0.11
Pop11	< 0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	< 0.01	0.01	<b>0.87</b>

## 4. Discussion

*Anax imperator* is a large dragonfly that probably has good dispersal abilities within pond networks. The  $F_{ST}$ -values and absolute  $D_{est}$ -values between the populations sampled in the Normandy region were all below 0.03 indicating a low level of genetic differentiation compared to previous studies on pond odonates at a local or regional scale (i.e.  $F_{ST}$  up to 0.08, 0.10, 0.24 and 0.28 for *Leucorrhinia dubia*, *Coenagrion scitulum*, *C. mercuriale* and *L. dubia* respectively; Keller et al. 2010; Swaegers et al. 2015; Lorenzo-Carballa et al. 2015; Dolný et al. 2018). This result confirms the high mobility of *A. imperator* between ponds at the regional scale. However, at the European scale, some populations presented a moderate level of genetic differentiation, especially the population sampled in the United Kingdom that presented higher genetic differentiation.

All populations presented an observed heterozygosity close to expected levels, except the population sampled in the U.K. In this population, observed heterozygosity was lower than expected heterozygosity and allelic richness was lower compared to other populations. This low genetic diversity was also associated with significant degree of inbreeding. Low genetic diversity and inbreeding often indicate a previous population bottleneck or the founder effect of a recent colonisation (Allendorf 2013). In the current context of climate change (IPCC 2013), many species are shifting their distributions to higher altitude or toward the poles (Hickling et al. 2006). A northward shift of range margins was already reported for the distribution of many odonates in England, including *A. imperator* in England that moved 85 km to the North between the period 1960–1970 and 1985–1995 (Hickling et al. 2005). For this study, the U.K. population of *A. imperator* was sampled near the city of York, at the Northern margin of its current distribution range. The observed low genetic diversity might therefore be a consequence of a recent colonisation of these ponds. The two French populations from Heudreville and Bois-Guillaume also presented a significant degree of inbreeding. This result is quite unexpected because these two populations were among the largest that were sampled in the Normandy region. The genetic variation was much higher within populations than between them. No significant variation among French populations was detected, indicating that they all have a similar genetic diversity in Normandy region. However, a significant variation was also found among countries. This significance is probably due to the UK population that presented a moderate genetic differentiation with all other populations.

Increasing genetic isolation with distance is a common relationship that often shapes the genetic structure of populations (Sexton et al. 2014). At the European scale, this pattern was already

reported for dragonflies (*Ischnura elegans*; Wellenreuther 2011), but also other flying insects (*Operophtera brumata*; Leggett et al. 2011) or flying mammals (*Myotis daubentonii*; Atterby et al. 2010). In this study, no pattern of isolation by distance among the populations of *A. imperator* was found at the European scale (Fig. 5.2). However, three main groups of populations were identified with STRUCTURE and GENELAND. These groups were roughly distributed as follows: Eastern populations, Normandy populations and the U.K. population. One Normandy population (*i.e.* Beaussault) had a genetic diversity very close to the Eastern populations, especially the Swiss population. Some Normandy populations were only receivers of gene flow. This indicates that they may act as sink for the species, whereas other populations were detected as source populations. Especially there was a significant directional gene flow from the population of Cerisy to many other Normandy populations and to all Eastern populations. Overall, the results indicate a high gene of *A. imperator* between all sampled populations from continental Europe. These populations may therefore be connected either by long distance movements (Suhling et al. 2017), high rates of short distance movements between ponds, leading to stepping stone dispersal (Saura et al. 2014), or both.

Although it was not geographically very far (ca. 500 km), the U.K. population presented a higher genetic differentiation with the Normandy populations compared to the other European populations (Fig. 5.2). This result suggests that the English Channel could act as a physical barrier to the gene flow of *A. imperator* (Razgour et al. 2014). Nevertheless, one Normandy population (*i.e.* Bresle) received individuals from the U.K. population. This can be explained by dominant NW winds brought by the gulf stream that may support occasional dispersal events and therefore make the U.K. ponds as a source for some Normandy ponds. The lack of gene flow from Normandy to the U.K. is a bit surprising, since no genetic difference due to the English Channel or the Baltic Seas was found in the small species *I. elegans*. This difference in results for these both species may be due to the fact that the populations of *I. elegans* can reach very high densities at some site, which increased the observed intra-population variation and limit genetic drifts. On the contrary, the populations of *A. imperator* are smaller in comparison and may therefore be more prone to genetic differentiation due to the lack of gene flow (Allendorf et al. 2013).

Genetic studies on odonates mostly use fresh material, especially legs of adults (Lorenzo Carballa 2015; Troast et al. 2016) or sometimes heads of adults (Chaput-Bardy et al. 2008; Wellenreuther et al. 2011). However, this collection method is invasive and should be avoided for species with high conservation value (Monroe et al. 2010). Alternative methods based on DNA extraction from exuviae are therefore increasingly used (Keller et al. 2010; Dolný et al. 2018). This

non-invasive method has the advantage to ensure that the individuals have grown in the studied site, while the origin cannot be always assessed for adults (Raebel et al. 2010). For large species, collecting exuviae is also easier than catching flying adults that fly very fast over the ponds in their reproduction sites. Nevertheless, the persistence of DNA in these chitin envelopes is poorly known. Especially, enzymatic action on hydrated exuviae and prolonged exposure to sunlight may lead to a significant reduction of DNA yields (Watts et al. 2005). In this study, we compared the DNA yields of fresh exuviae reared at the laboratory with other 'old' exuviae sampled in situ. Although the total amount of DNA was much lower in 'old' exuviae, most samples (*i.e.* 68.4 % of 'old' exuviae versus 89.9 % from 'fresh' exuviae) could be used for this microsatellite study. We therefore recommend this method for further studies, at least on large species that are likely to contain more genetic material.

Legs of larvae provide also a reliable source of DNA, but are still seldom used in population genetic studies on dragonflies (but see Monroe & Britten 2014; Kohli et al. 2018). Contrary to adult or exuviae, larvae can be sampled by all weather conditions and during nearly all seasons of the year, a feature that can simplify the schedule of field sessions. Moreover, in many species, high larval densities make it easier to collect a large number of samples, whereas flying adults may be hard to catch, especially Aeshnidae. Although the identification of some species may be difficult on the field, we suggest that larval DNA sampling could more applied in further studies, especially on large dragonfly species. It would also be interesting to test whether the removal of one tibia could significantly affect the survival of the individual.

Overall, this study provides insights into the spatial genetic structure of *A. imperator* populations at both regional and European scale. A high gene flow was found between continental populations, indicating that the distance between ponds at the European scale do not prevent dispersal movements of this large dragonfly species. The results also highlight the role of the English Channel as a potential barrier to dispersal, especially from Normandy to the U.K. Further investigations on other populations from southern England would be interesting to better understand the effect of this barrier on gene flow.

## 5. Acknowledgements

We want to thank the contributors that accepted to send us samples for this study. Thanks are especially due to Andrea Corso from Sicily, Sebastien Tschanz from Swiss and Martin Roberts from the United Kingdom.

# Discussion and perspectives

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This thesis work aimed to provide a transversal insight into the dispersal of dragonflies and study factors that affect its characteristics. Several aspects of dispersal were addressed, from the individual traits and local movements to the gene flow between populations. In particular, the colonisation of new ponds was first studied, the relationship between larval development and adult traits was then investigated and finally local movements and actual dispersal of the large dragonfly *Anax imperator* were assessed.

In this last part of the manuscript, the initial hypotheses are recalled and responses are provided, followed by a discussion to each of them. The relationship between the individual traits of dragonflies and their dispersal is then summarised. Finally, the implication of our work for pond network management is discussed, followed by perspectives for future work on dragonfly dispersal.

## Discussion on part I: Colonisation of new ponds by dragonflies

### Summary of the results

**Hypothesis I: The intrinsic dispersal capacities of dragonflies as well as environmental factors condition their ability to colonise new waterbodies.**

In Anisoptera, most colonising species arrived during the first year after pond creation and a low colonisation rate was found in subsequent years. In Zygoptera, most species also arrived the first year, but the colonisation rate was still high in the second year and decreased the third year. This difference in colonisation patterns between Anisoptera and Zygoptera shows that Anisopteran species are somewhat more responsive to the creation of new ponds than Zygoptera. This result may be explained by the fact that Anisoptera are generally more robust and have larger wings than Zygoptera (Suhling et al. 2015). They have therefore better flight abilities which facilitate movements around waterbodies (Conrad et al. 1999; Suhling et al. 2017) and colonisation of new ponds. The landscape context, especially a gradient from small forest ponds to large ponds in open areas, was the first variable shaping odonate communities. There was also a shift in the community composition over time, with generalist species presenting more occurrences at the beginning of the survey, whereas occurrences of forest specialists increased with pond age. Surprisingly, during the first years, the vegetation structure only had a low impact on species assemblages. The total odonate abundance was related to the structural connectivity between ponds, and this relationship was especially true for Zygoptera that have lower dispersal abilities. The structural connectivity also played a role in the composition of species assemblages and especially, the occurrences and abundances of generalists and specialist species of open landscapes increased with the density of ponds in the surroundings. This result could be due to metapopulation dynamics that allow a better population maintenance when ponds are connected in a network (Hanski & Ovaskinen 2000; Thornhill et al. 2018).

Overall, the colonisation pattern of new ponds differed slightly according to the suborders and the ecological groups of species. While the vegetation structure had a low effect on community structure, the landscape context and pond connectivity shaped species occurrence and abundance on new ponds. **The hypothesis I is therefore validated.**

## Methodological comments on the identification of larvae

Specimen identification was based on a key for all larvae of Northern Europe (Norling & Sahlén 1997) in association with another key for anisopteran larvae of middle Europe (Müller 1990). However, some species were not covered by these keys and identification was therefore completed by two keys initially intended for the identification of exuviae (Heidemann & Seidenbusch 2002; Brochard 2012). We first started by identifying the final instars, that generally share the same characteristics as exuviae. We then inferred what criteria could also be used in smaller instars by empirically comparing with other younger larvae from the same sample. After identification, the larval habitus and some morphological details were also compared to the watercolour paintings of P. A. Robert (Brochard, 2018). Altogether, we were able to identify 97.4 % of the larvae, even sometimes very small specimens (*i.e.* length < 5 mm) using the criteria presented in Appendix 1. Nevertheless, further taxonomical work is needed to find reliable criteria to distinguish between the species *Coenagrion puella* and *C. pulchellum*, *Sympetrum sanguineum* and *S. striolatum*, *Anax imperator* and *A. parthenope* at the larval stage.

## Species ecological niches

Generalists often have better dispersal abilities and can therefore occupy habitat patches left by specialists even under low spatial connectivity (Nagelkerke & Menken 2013). Their abundance is therefore strongly related to the number of sites occupied (Verberk et al. 2010). On the contrary, in a well-connected network of favourable habitats, specialists are more competitive and can lead to the extinction of generalists (Nagelkerke & Menken 2013). The distinction between generalists and specialists is therefore a crucial aspect of species ecology to understand dispersal patterns of species.

In our study, several taxa were grouped due to issues in identification to the species level. However, these groups might have masked a specific pattern in species ecology. For instance, based on the regional occurrences, we could expect *C. puella* to be more generalist than *C. pulchellum* in Normandy (CERCION 2017). According to a study from Northern Europe, *S. sanguineum* might be a forest specialist whereas *S. striolatum* is likely to be a more generalist species (Kokoramäki et al. 2018). However, the ecological niche of species can vary within its distribution range according to the latitude. In fact, many species tend to be more generalist in the middle of their distribution range and more specialized at their range margins (Oliver et al. 2009, Sexton et al. 2017). Thus, a comparison of ecological niches of the species found in Normandy with previous published studies was carried out (Table 3). A total of 4 species presented a major difference in attributed ecological niche according to the studied region.

Overall, this result highlights the fact that ecological niches cannot always be inferred from previous studies and they should be considered at the regional scale for each study.

**Table 3.** Comparison of species ecological niches according to the studied region (i.e. Present work from Normandy, France; Sahlén 2006 from Scandinavia; Korkeamäki et al. 2018 from Scandinavia; Verberk et al. 2008, compiled from several studies across Europe). Abbreviations: “gen” for generalists, “spe” for specialists and “int” for intermediate species.

Genus species	Present work	Korkeamäki et al. 2018	Sahlén 2006	Verberk et al. 2008
<b>Anisoptera</b>				
<i>Aeshna affinis</i>				
<i>Aeshna cyanea</i>	<b>spe</b>	int	<b>spe</b>	<b>gen</b>
<i>Anax</i> sp.	spe	int	spe	
<i>Brachytron pratense</i>		spe		
<i>Cordulia aenea</i>		int		spe
<i>Cordulegaster boltonii</i>				
<i>Crocothemis erythrea</i>				
<i>Libellula depressa</i>	gen		gen	
<i>Libellula fulva</i>				
<i>Libellula quadrimaculata</i>		<b>gen</b>		<b>spe</b>
<i>Orthetrum brunneum</i>				
<i>Orthetrum cancellatum</i>	spe		spe	
<i>Orthetrum coerulescens</i>	gen	int		
<i>Sympetrum sanguineum</i>	-	gen	-	
<i>Sympetrum striolatum</i>	-	gen	-	
<b>Zygoptera</b>				
<i>Coenagrion puella</i>	gen	int	-	gen
<i>Coenagrion pulchellum</i>	-	int	-	
<i>Coenagrion scitulum</i>	spe		spe	
<i>Ceriagrion tenellum</i>				spe
<i>Chalcolestes viridis</i>	int		int	spe
<i>Enallagma cyathigerum</i>	spe	int	spe	
<i>Ischnura elegans</i>	<b>gen</b>	int	<b>spe</b>	
<i>Ischnura pumilio</i>	spe		spe	
<i>Pyrrhosoma nymphula</i>	<b>spe</b>	int	<b>spe</b>	<b>gen</b>

## Comparison between larval and adult inventories

During CMR and telemetry surveys on pond A in Bois-Guillaume (see Chapter 4 Fig. 4.1), the occurrence of dragonfly species was recorded. This 1 100 m<sup>2</sup> pond collecting runoff water from a highway, was converted into a permanent pond in 2013. Although no specific inventory protocol was applied for this survey, the final species list based on adult observations was rather exhaustive because the pond was visited at least once a week between May and September in 2017 and 2018. Larvae were also sampled on the springs of 2017, 2018 and 2019 following the protocol described in Chapter 1 (paragraph 2.2). The results of this three-year larval survey and two-year adults survey are presented in Table 4.

**Table 4.** Comparison between larval and adult inventories carried out on pond A in Bois-Guillaume (WGS84: 49.480, 1.102) in 2017, 2018 and 2019. The total number of larvae collected was reported and the presence of species detected at the adult stage is indicated with a “X”.

Family	Genus species	2017		2018		2019
		Larvae	Adults	Larvae	Adults	Larvae
Aeshnidae	<i>Aeshna affinis</i>				X	
	<i>Aeshna cyanea</i>				X	
	<i>Aeshna mixta</i>		X		X	
	<i>Anax</i> sp.	9	X	3	X <sup>a</sup>	
Coenagrionidae	<i>Ceriagrion tenellum</i>		X		X	1
	<i>Coenagrion gr puella</i>	57	X <sup>b</sup>	258	X <sup>b</sup>	79
	<i>Coenagrion scitulum</i>	2	X	13	X	17
	<i>Enallagma cyathigerum</i>	52	X	9	X	1
	<i>Erythromma viridulum</i>	2	X		X	29
	<i>Ischnura elegans</i>	110	X	224	X	49
	<i>Pyrrhosoma nymphula</i>		X			
Cordulidae	<i>Cordulia aenea</i>		X			
Libellulidae	<i>Crocothemis erythraea</i>		X		X	4
	<i>Libellula depressa</i>		X		X	
	<i>Libellula quadrimaculata</i>				X	
	<i>Orthetrum cancellatum</i>	8	X	16	X	3
	<i>Sympetrum</i> sp.	15	X <sup>c</sup>	71	X <sup>c</sup>	24
Lestidae	<i>Chalcolestes viridis</i>		X		X	
Total taxonomic richness		8	15	7	16	9

<sup>a</sup> one single male of *A. parthenope* was also found on one occasion in 2018

<sup>b</sup> only *C. puella* was found at the adult stage

<sup>c</sup> both species *S. sanguineum* and *S. striolatum* were found at the adult stage in 2017 and 2018

In average, the taxonomic richness detected at the larval stage represented 52 % of the richness detected at the adult stage. All species collected at the larval stage were subsequently found during the summer at the adult stage. However, many species seen at the adult stage were never found as larvae in the studied pond. These species were essentially Anisoptera (*i.e.* Aeshnidae, Cordulidae and Libellulidae) whereas Zygoptera, especially Coenagrionidae, were often found at the larval stage when adults were detected on the pond. This difference can be explained by the expected higher dispersal ability of Anisoptera that can visit waterbodies without breeding, whereas the less mobile Zygoptera tend to stay on the same ponds. It is also interesting to note that adults of *C. tenellum* and *C. erythrea* were observed in 2017 and 2018 but the larvae were first found at low densities in 2019. Although the larvae might have been missed in previous years, it is likely that these species could not breed due to a low adult density or the lack of suitable habitats before 2019. Our results confirm that adult inventories are less reliable because they tend to overestimate the species richness in odonates, since many species can be present without performing a full larval development in the pond (Raebel et al. 2010).

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## Discussion on part II: Larval development, consequences on adult condition and natal dispersal

The second hypothesis included a potential relationship between individual life traits and dispersal after emergence. However, chapters 2 and 3 did not address the question of this natal dispersal. Therefore, a complementary study (presented below) details the results obtained on reared individuals that were released after emergence during this PhD thesis work.

### Complementary study on natal dispersal

The maturation period remains one of the least known parts of odonates life. After emergence, dragonflies disappear in the surroundings of the ponds to forage and avoid all water surfaces until their maturation. In 1957, Corbet already stated that marking *A. imperator* at emergence on a large scale would permit to bridge the gap between exuviae (and thus larval traits) and newly emerged adults and mature populations. However, this has never been done *in vivo* on dragonflies, although it is expected that larval carry-over effect and the maturation period have a strong influence on survival and fitness of mature adults (Stoks & Córdoba-Aguilar 2012).

Telemetry systems allow to track dragonflies even when they are in terrestrial habitats away from the ponds (Knight et al. 2019; Le Naour et al. 2019). The most performant tools in telemetry are radio-tracking systems using active transmitters that can be tracked with mobile or fixed antennas and GPS systems that can now be monitored from space (Curry 2018). However, the battery mass of these transmitters (*i.e.* at least 0.2 g) only allows to equip the more robust organisms like birds, mammals or large insects (Kissling et al. 2014). Smaller and less powerful organisms still remain a challenge to track (Wikelsky 2007; Kissling et al. 2014). Harmonic radars and radio frequency identification (RFID) systems work with tiny passive tags (mass < 0.1 g; Kissling et al. 2014) that were also developed to study movements of some flying insects like bees (Capaldi et al. 2000, Barlow 2019), butterflies (Ovaskainen et al. 2008), or dragonflies (Hardersen 2007; Le Naour 2019). While they allow to identify individuals with unique codes, RFID systems have a very limited detection range (*i.e.* up to 8 meters; Kissling et al. 2014). On the contrary, harmonic radar systems only work in a single frequency but enable the detection of the tag up to ca. 50 m (Le Naour 2019). They do not allow to distinguish individuals but they are better adapted to the tracking of mobile insects, such as odonates, in the surrounding matrix of the ponds.

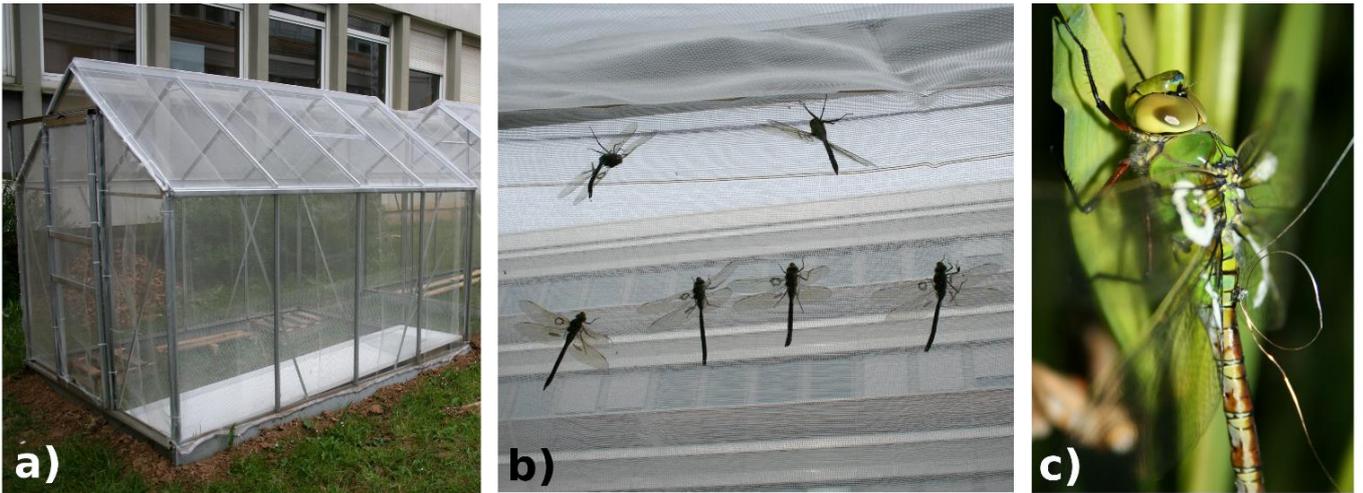
For this study, the 87 individuals (*i.e.* 43 males and 44 females) of *Anax imperator* previously reared (see Minot et al. 2019; Chapter 2) were marked and released on a single pond. All individuals were identified with a unique code on the wings and 38 females were also equipped with a passive transponder. Five ponds, including the releasing site and 4 ponds in the surroundings, were then monitored to record marked individuals.

The individuals that were marked in this study were the same that we reared for the study presented in Chapter 2. A least one hour after emergence, all individuals were marked with an alphanumeric code on the wings using white nail polish diluted to half strength with commercial acetone. The imagines were placed in a 2 × 3 m enclosure for their cuticle and wings to harden until the night (Fig. 13a & b).

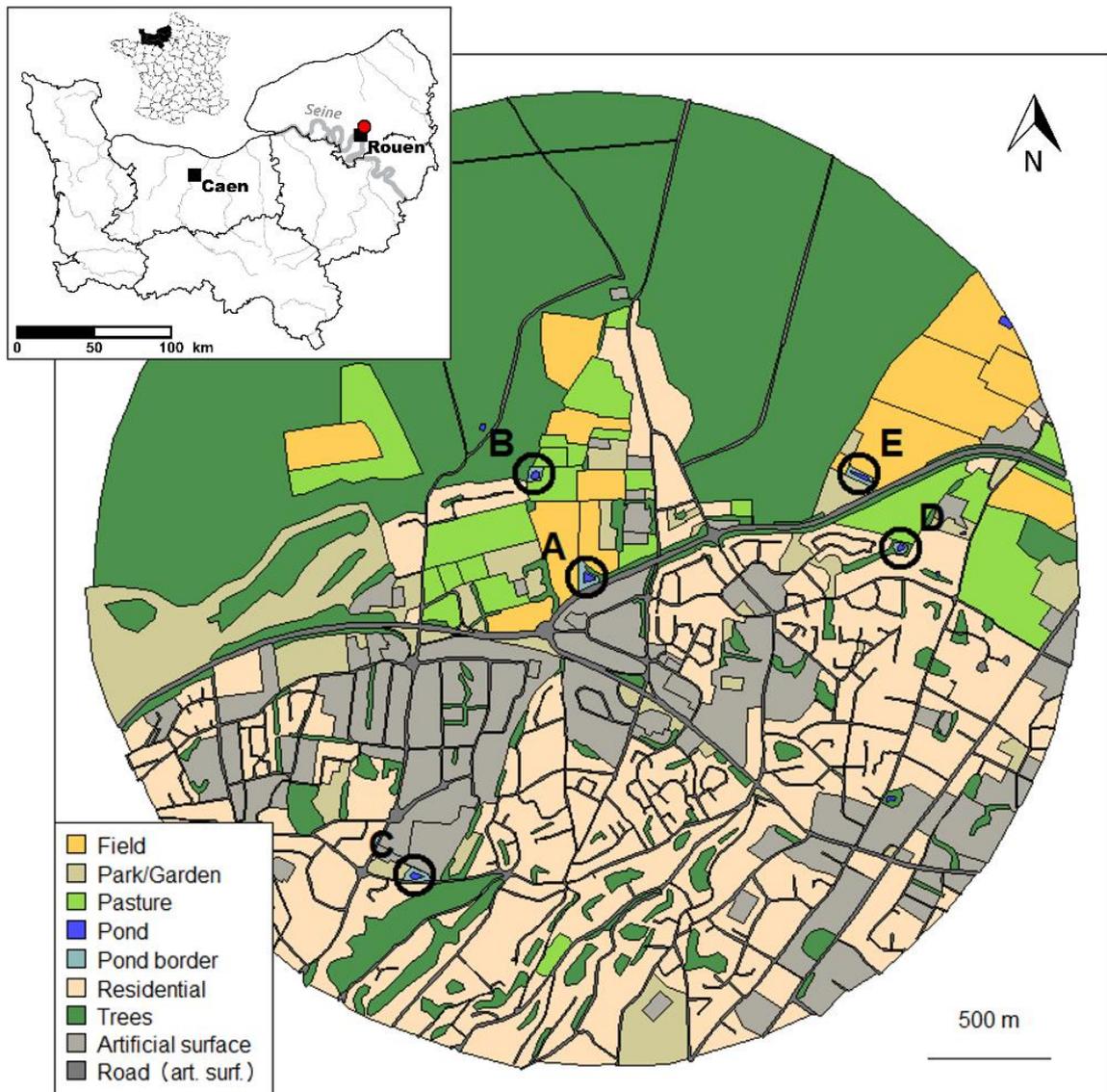
After sunset, females (*i.e.* a total of 38) were placed into modified a box that immobilised their wings and were equipped with a small tag on the dorsal side of the second abdominal segment (Fig. 13c). The tag consisted in a Schottky diode welded to an 8 mm wire antenna. A DC loop between the two poles of the diode was also added to preserve the diode from electrostatics charges (Hardersen 2007). Attachment of the tag was done with cyanoacrylate glue (Loctite®, superglue) combined with neoprene glue. Since males were expected to be easier to detect on ponds, they were not equipped with tags and only marked with an alphanumeric code on the wings. All individuals were then released at night (*i.e.* after 23:00) on a single pond (pond A; Fig. 14). They were all placed on the leaves of a yellow iris (*Iris pseudacorus*) located on the Eastern shoreline of the pond.

Two additional individuals were also equipped with radio-transmitters (Advanced Telemetry Systems, model A2412; frequency range 148-152 MHz; 15 bpm; 0.2 g) and released on the same site (see details on radio-transmitter attachment in Chapter 3; paragraph 2.3).

Five large and sun-exposed ponds were surveyed at least once a week between 31 May and 7 September 2017. All ponds were located within a radius of two kilometres from the releasing pond A (Fig. 14). Field sessions were performed between 10:00 and 17:00 under favourable weather conditions (*i.e.* temperatures above 20°C by sunny weather or above 25°C by cloudy conditions). Ponds and their immediate surroundings were scanned using a RECCO® detector at least once a week in order to detect females equipped with a tag. A visual inspection of pond banks and water surface was also done using binoculars to seek for males marked on the wings.



**Fig. 13.** Photography of the enclosure used for imago (a), six teneral individuals before their release (b) and immature female *A. imperator* after release at night (c). This individual was marked on the wings and equipped with a tag on the second abdominal segment.



**Fig. 14.** Localisation of the study area in France (i.e. red circle) and detailed map of the eight habitat classes (see Table 2.S1 in Chapter 2 for details) in the area delimited by a 2 km radius around the pond A. Ponds A, B, C, D, and E used for the study are circled in black.

Only two males marked with an alphanumeric code on the wings were found again after maturation on the 13/05/17 and 23/06/17 respectively. They were both resighted for the first time on the releasing site 18 days and 49 days later respectively. The first individual was seen again on the next day and never after. The second individual moved to pond B located 450 meters to the north 5 days after the first resighting and stayed there during 10 days until it was never resighted again. The other individuals that were never resighted might either have been missed, be dead, or have dispersed away from the study area. Since no other individual was resighted on the other ponds, we could not estimate the proportion of individuals that moved between ponds during the maturation period.

No female was found again after release during this study. Given the low recapture rate of males, it is possible that females were simply not detected because they were not marked in a sufficient number (*i.e.* 44 individuals). However, one tag was found on the ground about 20 meters far from the release point but no evidence of individual mortality was found. This finding supports the hypothesis that the tags may have detached from dragonflies during the maturation period. Indeed, the dorsal cuticle of *A. imperator* at emergence is covered with a layer of wax that made the attachment of the tag a bit difficult. The lack of resighting for females may also be linked to a higher mortality of the dragonflies equipped with tags. The length of the antenna and the wire loop might hinder the dragonfly movements, especially when it tries to hide in the vegetation. The harmonic tag attachment might therefore have an impact on survival. Further investigations on the effect of tag attachment under laboratory conditions would be necessary before further use.

The two individuals equipped with radio-transmitters were not able to take off. It is probably due to the low strength of individuals at emergence and it confirms that radio-transmitters weighing 0.2 g or more cannot be used to study odonate dispersal at emergence.

## Summary of the results

**Hypothesis II: Anthropogenic and environmental perturbations during the larval development can affect adult condition, survival and dispersal.**

In this work, 87 last instar larvae of *A. imperator* and 107 larvae of *A. cyanea* between F-1 to F-3 were reared until the emergence of the adults. Results highlight that the condition of the larvae, especially the total length of the exuviae in *A. imperator*, was strongly related to the mass and length of individuals at emergence. In *A. cyanea* larvae, the tibia length was also correlated between larvae up to F-3 and adults. It shows that adult condition may be inferred from larval traits even several stages before their emergence. The relationship between life traits and survival during the maturation period could not be established, since only two individuals were resighted during the survey sessions in 2017. Moreover, these individuals marked at emergence and resighted after the maturation period were found on their releasing pond. The hypothetical natal dispersal could therefore not be studied here.

We found no relationship between the level of Hsp70 protein in larvae and the exposure to different concentrations of Round-Up or DEET in the water. We expect that this lack of response is due to the fact that the model species is probably highly tolerant to water pollution at the considered concentrations (*i.e.* up to 30 mg.L<sup>-1</sup>). The morphological traits of the larvae did not change according to the concentration of Round-Up or DEET in the water. In the same way, this lack of response might be explained by the high tolerance of the studied species to water pollution at the considered pollutant concentrations. We did not detect any change in the condition of teneral adults according to the concentrations of Round-Up or DEET at which larvae had been exposed. However, the level of Hsp70 protein was significantly higher in the head of adults than in the head of larvae. In *A. cyanea*, the stress due to the emergence process seems therefore predominant compared to the potential stress induced by an exposure to water pollution during the larval stage, and especially at the concentrations used in our study.

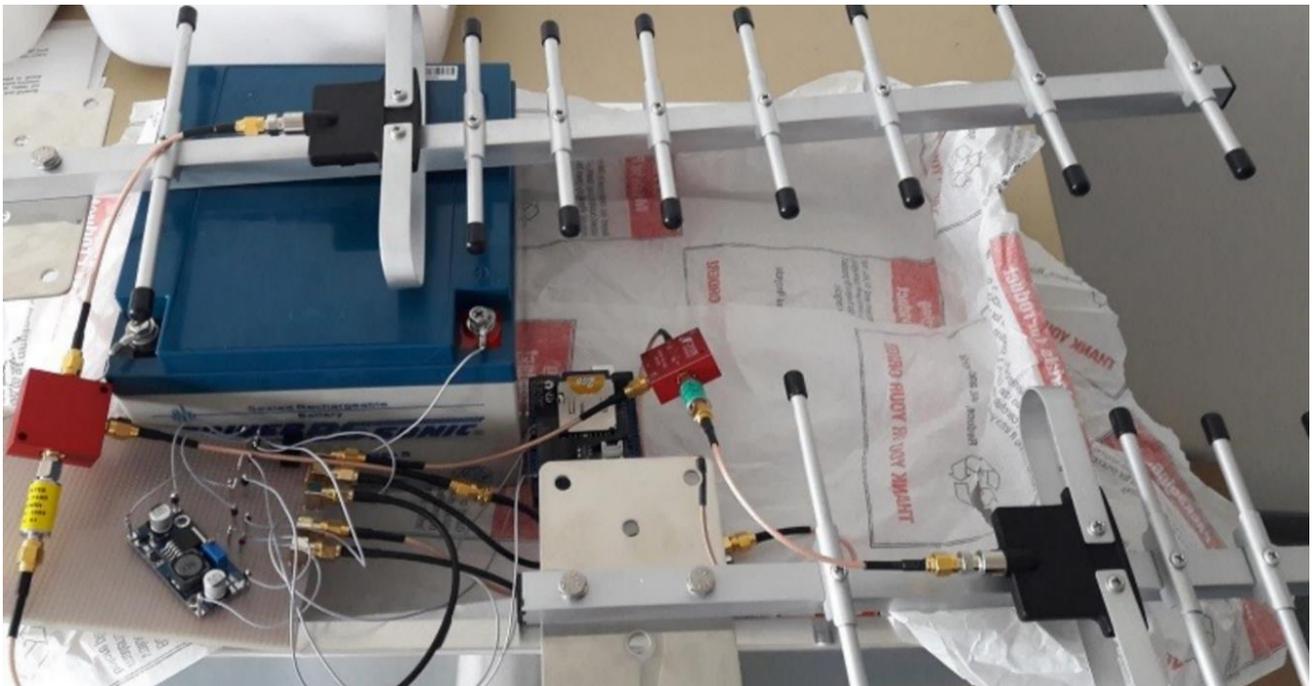
Overall, our results showed a strong relationship between larval life traits and the condition of adults at emergence. However, no effect of water pollution on individual traits could be detected and the relationship between these traits and natal dispersal could not be studied.

**The hypothesis II is therefore partially validated.**

## Methodological comments on harmonic radars

In this study, harmonic radars were used to try to detect teneral dragonflies in the terrestrial habitats and quantify their natal dispersal at emergence. However, the restricted detection range (*i.e.* about 50 m) and duration of the survey using a mobile detector was not sufficient to find back the females that were marked at emergence. Moreover, transmitter attachment on the dorsal part of the second abdominal segment was difficult and many individuals might have lost their equipment soon after releasing. Further tests on tag attachment should be made before using this method for further studies on *Anax imperator*.

To obtain a continuous monitoring of ponds and increase the chances to detect females marked after emergence, a project for the construction of fixed detectors was launched in 2017. The initial project aimed to develop fixed harmonic radar stations able to detect the presence of dragonflies equipped with a tag. These stations set on each pond were expected to perform a continuous monitoring and record the precise time and date at which dragonflies visit the ponds (Fig. 15). Unfortunately, this system could not be finished before the beginning of the field sessions due to issues encountered in amplifying and filtering the signal. The reflection of the signal by the tag (*i.e.* Schottky diode and antenna) could only be detected at distances no more than 1 or 2 meters away from the radar system and parasitic signals often prevented reliable detections. Many technical improvements are thus needed to enable the deployment of this system in the field.



**Fig. 15.** Photograph of a fixed harmonic radar system during laboratory trials.

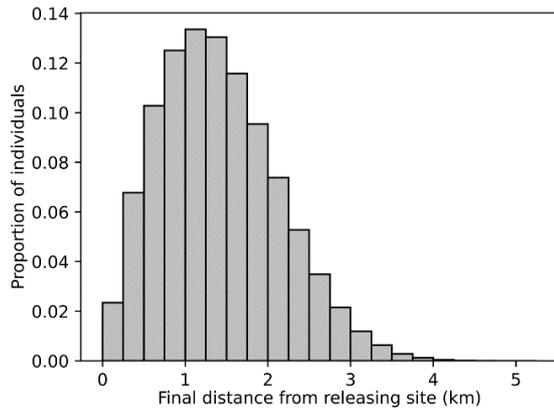
## Discussion on natal dispersal

One of the main gaps of studies on dragonfly dispersal is the natal dispersal performed by the young imagines during their maturation period (Hardersen 2007; Le Naour 2019). They spend up to 15 days foraging away from their natal pond and their elusive behaviour during this period makes them hard to study (Corbet 1957). In this work, we tried to address the question of natal dispersal by rearing and marking 87 individuals of *A. imperator* after emergence. Unfortunately, no female could be found again in the immediate surroundings of the ponds and the limited detection range of the material did not allow to detect them far in the matrix. Concerning males, the two individuals recaptured on their natal pond did not bring much information except that there is either a low philopatry, a high mortality, or a combination of both in this species (Knaus & Wildermuth 2002). This proportion of recapture (i.e. 5 %) is however in the same order of magnitude as previous studies on natal dispersal in dragonflies (cf. Fig. 12 from the general introduction).

Since dragonflies do not show any tropism toward ponds during their maturation period (Corbet 1957; Le Naour 2019), we might hypothesize that their movements follow a typical dispersal distance function (Clobert et al. 2001), that can be modelled by steps in random directions within the landscape matrix. The dispersal distance during the maturation of dragonflies would therefore only depend on the daily distance covered and the duration of this period (i.e. 10 days for males and 15 days for females; Corbet 1957). Based on the assumption that immature dragonflies can move 500 m on average per day a mathematical simulation was performed (Fig. 16). This model predicts that 9 % of the males would remain within a radius of 500 meters around their natal pond after 10 days of maturation (Table 5). This theoretical prediction matches with our observation since 5 % of males were found again on their natal pond. Considering that many individuals may not have survived during the maturation period, this order of magnitude seems coherent with the simulation. The model based on a random distribution would also predict a high number of males (i.e. 70 %) on the other ponds surveyed within a radius of 500 m – 2 km (Table 5). However, in our study, no dragonfly was found on other ponds at the end of the maturation period. Our dataset does therefore not support the hypothesis of a random distribution for the natal dispersal of *Anax imperator*.

Conrad et al. (1999) found no effect of sex or age (i.e. immature or mature) on the tendency of individuals to disperse in several species. However, another study on the Anisoptera *Leucorrhinia pectoralis* found a higher probability of movements between ponds in individuals marked at emergence (18 % of the recaptured individuals) than in mature individuals (0.8 %; Minot 2016). A study on

*Gomphus lucasii* also showed that the average moved distance was much higher during the maturation period ( $181 \pm 239$  m) than during the mature life ( $596 \pm 5$  m; Zebesa *et al.* 2015). These results suggest that natal dispersal could be more important than breeding dispersal at least in some dragonfly species. Further field studies are needed to better understand the natal dispersal and mechanisms occurring during this crucial period in the life of dragonflies.



**Fig. 16.** Proportion of individuals for each class of distance moved using a dispersal distance function. This representation shows the theoretical final distribution of 1 000 000 movements of 500 meters in random directions during 10 days.

**Table 5.** Theoretical dispersal during the maturation period of males (10 days) and females (15 days) based on the assumption that their movements follow a random distribution in the matrix with a mean daily distance of 500 m.

Distances from the releasing pond	Theoretical proportion of movements	
	Females	Males
< 500 m	0.06	0.09
500 m – 2 km	0.59	0.70
> 2 km	0.35	0.21

## Discussion on part III: Dispersal of the large dragonfly *Anax imperator*

### Summary of the results

**Hypothesis III: The characteristics of local movements and large-scale dispersal of dragonflies depend on both species' intrinsic and environmental factors.**

The telemetry survey on *Anax imperator* showed that females used a larger home range than males. The maximal distance moved from the releasing pond was also significantly higher in females than males. Indeed, males stay often closer to the ponds because they exhibit a territorial behaviour. On the contrary, females spend more time exploring the surrounding terrestrial matrix between oviposition sessions. They may also need more feeding resources to support the development of their eggs. Although females were more mobile in the landscape matrix than males, the rate of movements between ponds was not significantly different between males and females in the CMR models. This lack of difference between male and female movements may be explained by the fact that most movements detected by the CMR study were very short (*i.e.* < 500 m). They occurred between ponds that could be included in the home range of both males and females. Two longer movements (*i.e.* > 1000 m) between ponds were also detected, assessing that larger distances can occasionally be covered by this species. However, our data did not allow testing for any difference between males and females. As expected, individuals of both sexes had a strong tropism for ponds in which they breed. The tracked individuals, especially females, were also significantly more present in isolated large trees or forests than in other terrestrial habitats in the surroundings of the ponds. This result was not expected because *A. imperator* only breeds in large and sun exposed ponds, which are rare in forests. We expect that this species has therefore different habitat requirements to breed and rest. Trees may play a crucial role of shelter for dragonflies during the night or during unfavourable weather conditions.

The population genetic analysis highlighted a high gene flow between populations of *Anax imperator* in Normandy. This result emphasizes the high mobility of this species between ponds at the regional scale. Some ponds had however higher inbreeding coefficients than others, indicating that the rate of exchange may not be homogeneous between all ponds. At the European scale, 3 genetic clusters were found (*i.e.* U.K., France, eastern European countries). No geographical barrier due the presence of mountain chains like the Alps seemed to have an effect on the gene flow of *A. imperator* between French

populations and other eastern European populations, especially the Swiss population. However, the U.K. population sampled in the Northern range margin of *A. imperator* presented a genetic differentiation compared to the other sampling sites. The genetic diversity was also lower in this populations compared to others. No gene flow was detected from the Normandy ponds toward the U.K., but one pond in Normandy presented a recent immigration from the U.K. population. Therefore, our results suggest that the English Channel might limit the gene flow between Normandy and U.K. populations and acts as a barrier to dispersal. The observed unidirectional gene flow from the U.K. population could be explained by passive dispersal events due to the wind currents of the Gulf Stream, from North-West to South-East.

Overall, local movements of *A. imperator* depended on the sex, females being more mobile than males. They were also driven by particular habitats like ponds used for mating and surrounding trees for resting. At the European scale, the English Channel was identified as a potential barrier to gene flow but the sex-biased dispersal was not tested here.

**The hypothesis III is therefore partially validated.**

## Methodological comments on the marking methods

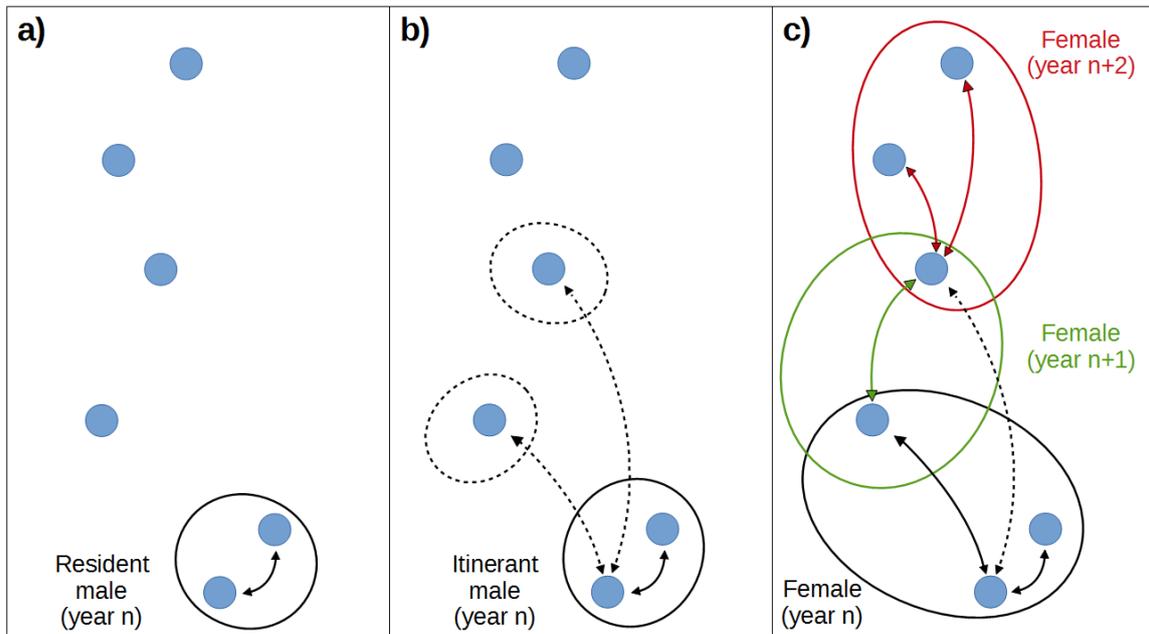
Wing marking is the simplest and the most cost-effective method to identify individuals and record resightings on waterbodies. Although reading codes on the wings of flying adults may be sometimes tricky through binoculars, the territorial behaviour of *A. imperator* males makes them stay enough time for a reliable identification in most cases. To facilitate the code reading, we used both white nail polish and a black permanent marker. The white marks were easier to read when dragonflies were flying above a dark background and the black marks were easier to read when they were flying up in the sky. This marking method has the disadvantage to be restricted to the ponds because dragonflies are very seldom found by chance in the surrounding terrestrial habitats. It is therefore well adapted to study the breeding behaviour of males (*i.e.* recapture rates  $> 0.50$ ), but insufficient to study behaviour of females that are more elusive (*i.e.* recapture rates  $< 0.25$ ). Since movements between ponds can be hard to detect, only marking a large number of individuals allow studying dispersal but it can be very time consuming. Finally, this method does not allow to find dragonflies when they are resting in the surroundings of the ponds or foraging away.

Radio-tracking was a very efficient method to track dragonflies in terrestrial habitats. A precise location was sometimes hard to get when individuals were high in the forest canopy due to the presence of branches disturbing the signal. Nevertheless, only this method allowed to identify places in which they were resting such as trees. The most critical part of the tracking was to get the tag fixed because dragonflies often tried to remove it with their legs before the glue dries. Maintaining the tag using a thin medical strip has proven very efficient to maintain the transmitter while the glue is drying. This method enabled us to collect a dataset on the use of terrestrial habitats by *Anax imperator*, however many individuals were also lost during the first days of tracking. Only a continuous monitoring of each individual might allow tracking all their movements even on long distances. However, even by favourable weather conditions, their movements are very unpredictable. They can stand still during several hours before taking off, and then move very fast in any direction. Thus, such a study of continuous monitoring would be very time-consuming but also very difficult to apply given current technologies available for insect tracking.

As a general rule in dispersal studies, the maximal distance observed depends on the size of the studied area (Franzén & Nilsson 2007). In this study, the total prospected area was 12.6 km<sup>2</sup> (*i.e.* circle with a radius of 2 km). Since many individuals were lost, we cannot exclude that they dispersed outside the range of the study area. The dispersal abilities of *A. imperator* were therefore probably greater than the extent of the study area. That is why our work focused more on local movements between ponds and the use on the terrestrial matrix, rather than actual dispersal distance. The distances reported in CMR studies also depend on the number of recaptured individuals (Hassall & Thompson 2012). In this study on local movements, a total of 87 individuals of *Anax imperator* were marked on the wings. This number was limited by the number of individuals present in the ponds and the difficulty to catch this species. It might seem quite low compared to previous studies on smaller species like *Sympetrum spp.* in which several thousands of individuals can be used (Watanabe et al. 2004; Hykel et al. 2018, 2019). However, the low number of individuals was compensated by a high recapture rate of males (*i.e.* > 0.50) and the use of radio-transmitters that enabled us to record many local movements in the terrestrial matrix for each individual. Indeed, the number of radio-tracked individuals for this study (*i.e.* 54 individuals) was very high compared to previous studies using telemetry on dragonflies (5 ind. in Levett & Walls 2011; 14 ind. in Wikelsky et al. 2006; 7 ind. in Moskowitz & May 2017; 38 ind. in Knight et al. 2019). It allowed us to quantify for the first time the mortality induced by radio-telemetry on dragonflies.

## From local movements to dispersal during the mature adult life: a female biased dispersal?

Ponds are scattered habitats in the landscape matrix that can constitute different breeding sites for dragonflies. Movements of individuals between nearby ponds are essential for the maintenance of populations via metapopulation processes. These movements may not always be considered as a real dispersal, since we showed that a single dragonfly can use several ponds within the same home range and move regularly between them during its life. Nevertheless, the offspring of these individuals may in turn disperse further to other neighbouring ponds. Across several generations, successive movements between habitat patches may therefore result in a significant gene flow by a process of stepping-stone dispersal (Saura et al. 2014). Stepping-stone models were showed to give better prediction of dispersal over wide spatial and temporal scales than models considering only direct dispersal (Saura et al. 2014). Although it was not studied yet, we suggest that these stepping-stone movements across generations are very important in dragonflies because these species have a short life but move frequently between ponds and produce large offspring. Especially, females are more likely to generate dispersal according to this process, since we showed that they were more mobile in the landscape matrix than males. Mature males of *A. imperator* had a smaller home range than females, probably because they aim to defend a territory on a pond to increase their chances of mating. The males that succeed in establishing on a territory and breeding, are called residents. They are likely to stay on the same pond during all their mature life (Fig. 17a; Kasuya 1997). Other males, called itinerants, do not succeed in establishing on a territory. They will probably fly away to find another pond in their home range or further away, but they may not be able to breed there either (Lancaster & Downes 2017). Such movements may lead to actual dispersal but this dispersal will be effective only if they succeed in breeding on the new pond (Fig. 17b). Contrary to males, females are able to breed on any pond where environmental conditions are favourable because territorial males are waiting for them. Moreover, since the effects of fecundation can last several days in *A. imperator*, females can then either oviposit in the same pond or move to other ones within their home range (Corbet 1957). This process might not be called dispersal because they stay in the same home range. However, since the home ranges of females are very large, their offspring might in turn include several other ponds in their home range and generate a gene flow at the landscape scale over several generations (Fig. 17c). As for males, this process might also be completed by occasional dispersal events on longer distances which mechanisms remain poorly known in dragonflies (Rouquette & Thompson 2007; Keller et al. 2010). Overall, we expect that frequent local movements, even at the scale of the female home range might be responsible for a high gene flow between populations compared to occasional dispersal events on longer distances.



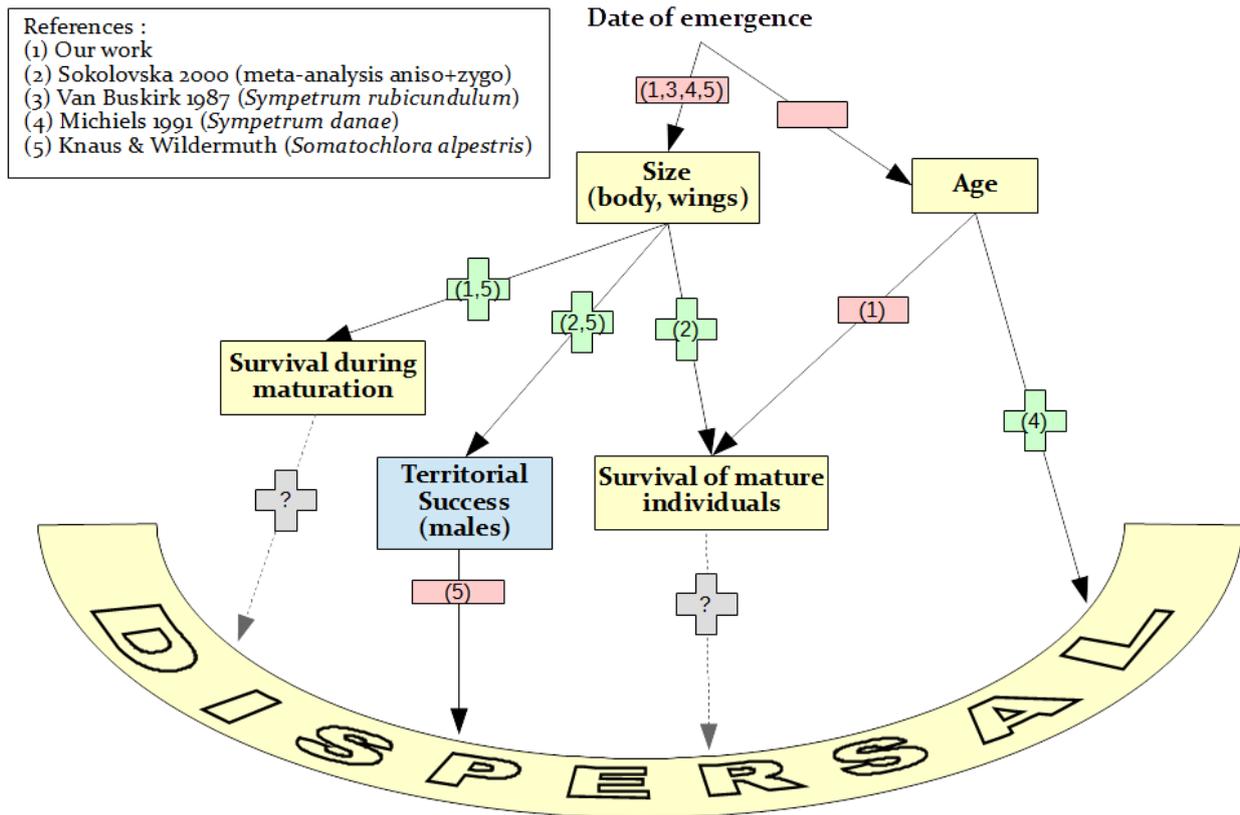
**Fig. 17.** Schematic representation of local movements within the home range (continuous arrow lines) and occasional dispersal events (dashed arrow lines). Blue dots represent the ponds and circles represent the home ranges of individuals.

Some previous studies suggest that, natal dispersal might be more important than breeding dispersal in dragonflies (Zebbsa et al. 2015). Although no general rule was established yet, the natal dispersal probably plays a determinant role in the global species dispersal that may overwhelm the outcome of the breeding dispersal. In this case, the potential sexual bias in dispersal would more dependent on dragonfly natal dispersal than breeding dispersal.

## General discussion and perspectives

### Linking life traits to dispersal

Life traits like body size can affect the dispersal abilities of individuals (Sekar 2012). Generally, individuals with a better morphology (*e.g.* large body size) or physiology (*e.g.* energy reserve) disperse in higher rate or over longer distances than their congeners (Clobert et al. 2012). In our work, we confirmed that the studied morphological traits of the larvae (*e.g.* head width, length of exuviae) along with the date of emergence were related to the traits of the adults. We do not know to which extent the differences in teneral traits could affect the dispersal at emergence. However, we found that mature adults tended to be larger than teneral adults, leading to the hypothesis that individuals emerging with larger bodies may be more likely to survive during the maturation period. During the mature adult life, no trait was found to be significantly related to the observed movements between ponds. However, survival was related to the wing loading and age. We might therefore expect that dragonflies with a lower body mass and larger wings will have better dispersal abilities, as well as more time to disperse during their life than their congeners. However, in territorial species, Knaus & Wildermuth (2002) stated that resident males had longer wings and were significantly less inclined to disperse. The relationships between individual traits and dispersal are summarised in Fig. 18.



**Fig. 18.** Schematic representation of individual traits and their consequences on dragonfly dispersal. Only studies on territorial Anisoptera were used here. Traits that are relevant for both males and females are displayed in yellow boxes and a trait that is only relevant for males is displayed in the blue box. Grey colour indicates a probable relationship, green colour indicates a confirmed positive relationship and red colour indicates a confirmed negative relationship.

## Connectivity and management of pond networks in Normandy

The colonisation of new ponds by odonates occurred very quickly after a pond creation or restoration and the abundance of larvae did not depend on pond age. These results suggest that most ponds are connected in networks at the regional scale and enable odonate populations to function as metapopulations with frequent dispersal events allowing colonisation and rescue effects. This hypothesis is also supported by the fact that occurrence and abundance of many species was also dependent on the density of neighbouring ponds. The study on the local movements of *A. imperator* also highlighted that this large species was able to move frequently between nearby ponds separated by 500 m. A previous work on the small zygoptera *Ischnura elegans* showed however that movements between ponds were not very frequent at the individual scale (Le Gall et al. 2017). Since the populations of this small species are often much larger than those of larger species (e.g. 383 *I. elegans* larvae and 12 *A. imperator* larvae collected in pond A; Table 4), even low movement rates at the individual scale may

allow connecting populations between several ponds. This hypothesis is confirmed by the population genetic study on *A. imperator* as well as a previous study on *I. elegans* (Le Gall 2016), which both showed a high gene flow between Normandy ponds. Moreover, according to the number of mature individuals caught on the ponds (*i.e.* max. 26 individuals) and the high recapture rate (*i.e.*  $> 0.50$ ), it can be inferred that the breeding population of *A. imperator* on the ponds was very small. The viability of such a breeding population over time is very unlikely without frequent exchanges between ponds and other neighbouring populations. Similarly, amphibians are also often organised as metapopulations (Smith & Green 2005; Bailey & Muths 2019). Most amphibian species do not disperse further than 1 km and 44% of the studies found maximum dispersal distances below 400 m (Smith & Green 2005). Therefore, a high connectivity between waterbodies is crucial to support a high biodiversity of freshwater species within pond networks (Ribeiro et al. 2011; Thornhill et al. 2018).

To maintain or restore connectivity in pond networks, the creation of new ponds can be very simple and, in most cases, not more expensive than pond restoration works after complete drying out (Williams 2008). In this work, we showed that newly created ponds were able to host a similar odonate diversity compared to restored ponds. In order to maintain a good connectivity between ponds at the regional scale, we therefore suggest that pond restoration should not always be prioritised over pond creation. The localisation of ponds is crucial for the life of inhabiting freshwater species because a good connectivity with other ponds facilitates dispersal events (Hill et al. 2018). The structure of the surrounding landscape can also affect pond communities. For instance, the proximity of transport infrastructures might have a significant impact on mortality (Šigutová et al. 2017; Cayuela et al. 2019) whereas the presence of nearby trees might provide a shelter for amphibians like salamanders (Piraccini et al. 2017) and dragonflies species like *Leucorrhinia caudalis* (Le Naour et al. 2019) or *A. imperator*. When taking these requirements into account, some old ponds might not be worth to restore, whereas the creation of new ponds at strategical localisations might have a very beneficial outcome for pond biodiversity at the regional scale. For practical reasons, the choice of the localisation might also be driven by the availability of land, the depth of groundwater or the local topography.

## Further challenges and perspectives for dispersal studies on dragonflies

### Quantifying natal dispersal

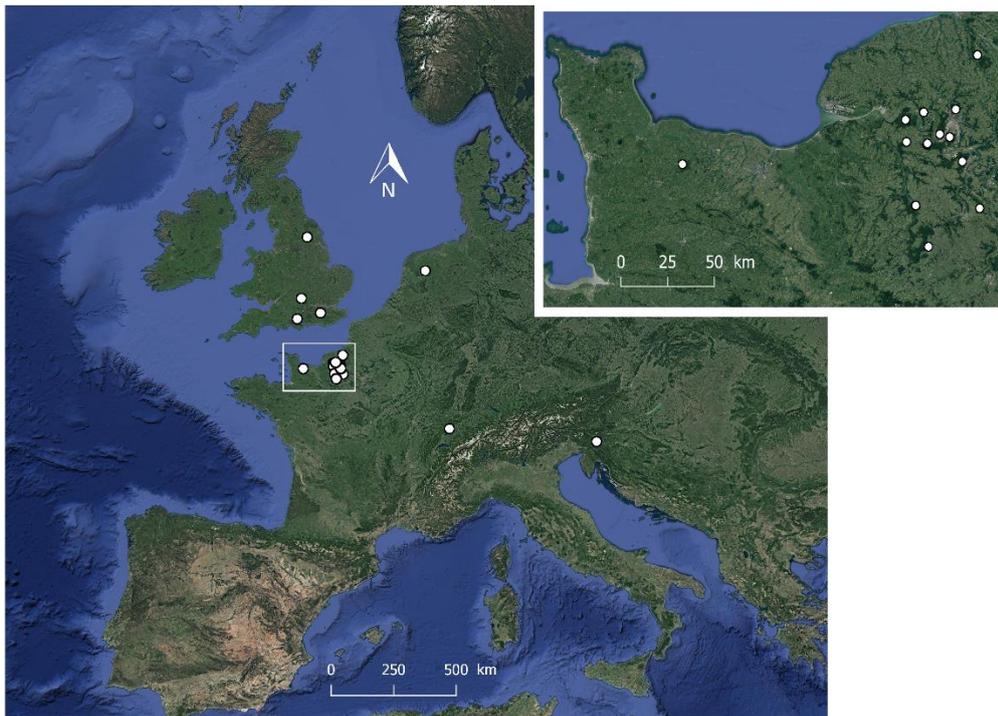
The main issue in quantifying the dispersal abilities of dragonflies is often the lack of information on their natal dispersal. Classical wing-marking studies can bring insights into the movements that occur during the maturation period. However, they are often very complicated due the behaviour of immatures to avoid waterbodies and the very low recapture rates when they come back after maturation. Recent advances in radar technologies now provide light passive tags that can be fixed on adult dragonflies after emergence. This method does not allow identifying each individual, but if all individuals are marked on the same pond, it can provide information on the dispersal from one site to the surroundings. Surrounding ponds might be equipped with fixed radars to detect the potential passage of a marked individual. Unfortunately, this radar system was not operational during the field sessions of our work, but its further development might help filling the gap on natal dispersal of dragonflies. The development of this method must however take into consideration a potential effect on survival of the tag in addition to the natural mortality during this critical period of the life of dragonflies. Therefore, marking a high number of individuals will probably be required in order to get enough resighting. One advantage of this method is the very low cost of tags (*i.e.* self-made for 1 € and purchased from RECCO for 10€ per unit) that enables a very cost-effective deployment of tags on a large number of individuals.

### Assessing a potential sex biased dispersal

A study based on mitochondrial DNA showed that the desert dragonfly *Trithemis arteriosa* presented likely a male biased dispersal between waterbodies in Namibia and Kenya (Damm & Hadrys 2011). In these deserts, the distance between waterbodies can only be covered by long distance dispersal. Mitochondrial DNA markers indicate that these movements are likely performed by males, whereas short distance movements of females do not allow for a regional gene flow. A similar study on mitochondrial DNA in our regional pond network would be interesting to compare the consequences of dispersal on a higher pond density on a potential sex biased dispersal. In particular, such a study on mitochondrial DNA could investigate the potential female-biased dispersal due the higher vagility of females in the landscape matrix that was observed in our study.

### Identifying potential barriers to gene flow

The genetic study of the populations of *A. imperator* highlighted a high gene flow between populations at the scale of the Normandy region and a potential effect of the English Channel acting as a barrier between Normandy and U.K. populations. *Aeshna cyanea* is another common Aeshnidae in Normandy that can be found in a large panel of ponds. This species has a preference for shaded forest ponds (cf. Chapter 1) in which larvae can be very abundant. This species is therefore complementary to *A. imperator* that breeds essentially in large sun-exposed ponds. It would be interesting to complete our population genetic study with this second species that have different habitat requirements. It would allow testing, among others, whether the difference in habitats used by these species can have an impact on gene flow at local and continental scale. We expect that forest habitats that are more fragmented than open areas across European landscapes will lead to a higher genetic differentiation. To address these questions, 13 sites from Normandy and 7 sites from 4 other countries were already sampled (Fig. 19). Among these sites, 4 are located in U.K and should also allow us a better understanding of the impact of the English Channel on the gene flow of large dragonfly species.



**Fig. 19.** Map showing the 20 populations of *Aeshna cyanea* that were already sampled.

*Simulating movements between ponds through a network model*

The connectivity between ponds working as a network is essential to host a larger abundance of dragonflies and allow population maintenance through metapopulation dynamics. The least-cost path method, based on the resistance of the landscape matrix, allows identifying the best path between two populations. Since the resistance of the matrix depends on the studied taxon, these models may be calibrated using the current data of the mobile anisoptera *Anax imperator* along with a dataset on the less mobile zygoptera *Ischnura elegans* collected during the project ROAD in Normandy (Le Gall 2016). Besides the structural connectivity between ponds, environmental characteristics also condition their ability to host different species. The data on species occurrence and abundance can be used to calibrate the probability of each pond to be colonised according to its characteristics. This could finally permit to assess the relative importance of each pond to provide connectivity within the network and simulate the effect of a pond loss on the overall network connectivity. Such a model at the Normandy scale would help identifying the potential threats on pond dragonfly populations and guide the management of pond networks, especially the works of restoration and creation of new ponds.

**Appendix: Criteria used for the identification of 23 dragonfly species found at larval stage in ponds of Normandy**

These criteria are based on Müller 1990, Norling & Sahlén 1997, Heidemann & Seidenbusch 2002, Brochard 2012, completed by some personal observations.

<i>Genus species</i>	Criteria used for identification
<b>ANISOPTERA</b>	
<i>Aeshna affinis</i>	<ul style="list-style-type: none"> <li>- Mentum: flat labium length / maximal width = 1.1 (may be only true for Fo larvae)</li> <li>- Coloration: light with dark spots on the dorsal side of the abdomen</li> <li>- Eyes: projected toward the anterior part of the head</li> <li>- Abdomen: presence of lateral spines on the 6<sup>th</sup> segment (S6) lateral spines of S<sub>9</sub> reaching more than the middle of S<sub>10</sub></li> </ul>
<i>Aeshna cyanea</i>	<ul style="list-style-type: none"> <li>- Mentum: flat</li> <li>- Coloration: generally dark labium length / maximal width = 1.5 (may be only true for Fo larvae)</li> <li>- Eyes: projected toward the anterior part of the head</li> <li>- Abdomen: presence lateral spines on the 6<sup>th</sup> segment</li> </ul>
<i>Anax imperator</i>	<ul style="list-style-type: none"> <li>- Mentum: flat</li> <li>- Eyes: very large and projected laterally (D-shaped)</li> <li>- Abdomen: absence of lateral spines on the 6<sup>th</sup> segment</li> </ul> <p><i>nb: young instars can be very hard to distinguish from A. pathernope. However, this species is scarce in small waterbodies of Normandy and all the last instars specimens in our samples were only A. imperator.</i></p>
<i>Brachytron pratense</i>	<ul style="list-style-type: none"> <li>- Mentum: flat</li> <li>- Eyes: projected toward the anterior part of the head smaller than the occiput (unique criteria)</li> <li>- Occiput: angular</li> </ul>

<p><i>Cordulia aenea</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped small line from the base and directed toward the anterior part (unique criteria, but sometimes hard to see)</li> <li>- Eyes: smalls</li> <li>- Abdomen: rounded</li> <li>- Legs: very long and slender</li> <li>- Thorax: crossed laterally by a dark stripe</li> </ul>
<p><i>Cordulegaster boltonii</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped labial palps with marked irregular crenulations</li> <li>- Eyes: small and protruding</li> <li>- Abdomen: very elongated and sharp</li> </ul>
<p><i>Crocothemis erythrea</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped</li> <li>- Eyes: large</li> <li>- Legs: slender</li> <li>- Abdomen: rounded no dorsal spines</li> </ul>
<p><i>Libellula depressa</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped crenulations of the labial palp smooth rounded</li> <li>- Eyes: small</li> <li>- Abdomen: elongated and sharp dorsal spines, except on S9 cerci length not reaching the half of the paraprocts</li> </ul>
<p><i>Libellula quadrimaculata</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped crenulations of the labial palp sharp and well defined</li> <li>- Eyes: small</li> <li>- Abdomen: elongated and sharp dorsal spines, except on S9 cerci length exceeding the half of the paraprocts</li> </ul>
<p><i>Libellula fulva</i></p>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped</li> <li>- Eyes: small</li> <li>- Abdomen: elongated and sharp dorsal spines, also present on S9 cerci length exceeding the half of the paraprocts</li> </ul>

<i>Orthetrum brunneum</i>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped labial palps bearing more than 5 setae</li> <li>- Eyes: small</li> <li>- Abdomen: elongated and sharp no dorsal spine</li> </ul>
<i>Orthetrum cancellatum</i>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped</li> <li>- Eyes: small</li> <li>- Abdomen: elongated and sharp dorsal spine on S<sub>4</sub>, S<sub>5</sub> and S<sub>6</sub> but not on S<sub>7</sub> cerci length exceeding the half of the paraprocts</li> </ul>
<i>Orthetrum coerulescens</i>	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped labial palps bearing less than 5 setae</li> <li>- Eyes: small and protruding</li> <li>- Abdomen: elongated and sharp no dorsal spine</li> </ul>
<i>Sympetrum</i> sp.	<ul style="list-style-type: none"> <li>- Mentum: spoon-shaped</li> <li>- Eyes: large</li> <li>- Legs: slender</li> <li>- Abdomen: rounded with dorsal spines (even very small instars)</li> </ul>
<b>ZYGOPTERA</b>	
<i>Ceriagrion tenellum</i>	<ul style="list-style-type: none"> <li>- Habitus: robust</li> <li>- Colour: often orange</li> <li>- Occiput: angular</li> <li>- Mentum: 1 seta (unique for this species)</li> </ul>
<i>Coenagrion</i> gr. <i>puella</i>	<ul style="list-style-type: none"> <li>- Head: covered with small black points (rare in other species)</li> <li>- Caudal gills: parallel borders and rounded at the end transversal line forming a small drop in the middle upper and lower spine rows on the lateral sides about the same length (caution: the difference length increases in younger instar)</li> </ul>

<p><i>Coenagrion scitulum</i></p>	<ul style="list-style-type: none"> <li>- Colour: often very light</li> <li>- Femora: usually without transverse dark stripe (contrary to other zygoptera) very thick</li> <li>- Caudal gills: leaf shaped covered with dark spot in there distal part mucronate at the end</li> </ul>
<p><i>Enallagma cyathigerum</i></p>	<ul style="list-style-type: none"> <li>- Shape: slender with thin legs</li> <li>- Femora: with a brown transversal stripe</li> <li>- Caudal gills variable according to the stage: F0 : leaf shaped with black transversal stripes Other stages: more elongated, often terminated by long white setae</li> <li>- mentum: small spine at the base of the terminal setae of the labial palps (always present but may require x100 magnification and low-level lighting)</li> </ul>
<p><i>Erythromma viridulum</i></p>	<ul style="list-style-type: none"> <li>- Abdomen: rows of spines on the ventral side of the 2 first abdominal segments (S<sub>1,2</sub>) often 2 black longitudinal stipes on the ventral side</li> </ul>
<p><i>Ischnura elegans</i></p>	<ul style="list-style-type: none"> <li>- Wing pads: distinct black spot at the base of each wing-pad (disappear at the end of the metamorphosis of F0)</li> <li>- Caudal gills: leaf shaped on row of spine on the lateral sides much longer than the other</li> </ul>
<p><i>Ischnura pumilio</i></p>	<ul style="list-style-type: none"> <li>- Habitus: robust</li> <li>- Wing pads: no distinct black spot at the base of each wing-pad</li> <li>- Legs: transversal dark stripe often absent on the femora</li> <li>- Caudal gills: can resemble the one of <i>I. elegans</i>, but often thinner with parallel borders often opaque with a white coloration</li> <li>- Abdomen: spines are not clearly arranged in row on the lateral carina of S<sub>6,7</sub> and 8</li> </ul>
<p><i>Pyrrhosoma nymphula</i></p>	<ul style="list-style-type: none"> <li>- Habitus: robust</li> <li>- Colour: often very dark</li> <li>- Occiput: angular</li> <li>- Caudal gills: well contrasted patterns of black and dark spots</li> </ul>
<p><i>Chalcolestes viridis</i></p>	<ul style="list-style-type: none"> <li>- Habitus: slender with long and thin legs</li> <li>- Mentum: basal hook of the labial palp clearly separated from the others</li> <li>- Caudal gills: elongated with parallel sides</li> </ul>

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