

# **Conservation ecology of the threatened damselfly *Lestes macrostigma***

**An integrated life cycle approach**



**Philippe Lambret**

Supervisors:  
Prof. Dr. Robby Stoks  
Dr. Patrick Grillas

Dissertation presented in partial fulfilment  
of the requirements for the degree of  
Doctor of Science (PhD): Biology

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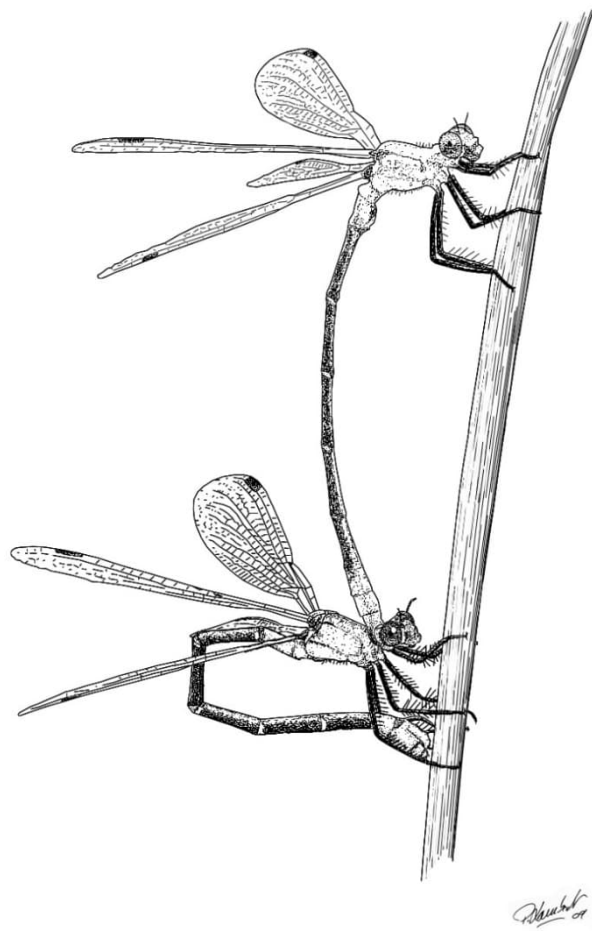
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Cover picture: Copulating wheel of *Lestes macrostigma* (© P. Lambret)

Back picture: Larva of *Lestes macrostigma* lying in wait (© P. Lambret)



*This work does not aim at exploring new fields of science or testing novel theories. It uses science to help stemming the erosion of biodiversity. This PhD thesis aims at feeding evidence-based conservation.*

*To my daughter. And all children who will heritate this changing world and have to face tremendous challenges. Good luck.*

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## Summary

Insects are the fastest declining group of species and among the most threatened by anthropogenic activities. However, they are largely overlooked in conservation policies and conservation research. Wetlands have greatly declined over the last centuries and continue to face important threats, impacting lots of organisms, including insects. Odonates (damselflies and dragonflies) are valuable indicators of wetlands' conservation status. Habitat restoration (including habitat recreation) has been raised as a priority strategy during the current decade. Such conservation actions are facilitated using flagship species, but to be efficient, a thorough knowledge of their ecological requirements is necessary. This doctoral thesis focused on the threatened damselfly *Lestes macrostigma*, as a flagship species to implement the conservation and restoration of temporary brackish ponds. There was limited knowledge concerning the influence of hydroregimes and water salinity levels on eggs and larvae, which are key stages in odonate population dynamics. With the goal of undertaking evidence-based conservation actions, we aimed to fill these knowledge gaps by conducting four studies in the Camargue, southern France. This area is characterized by salt deposits and a Mediterranean climate, creating brackish and temporary wetland habitats which harbour *L. macrostigma* breeding populations.

To improve our knowledge of the egg biology, we studied the embryonic development and hatching phenology and success in two temporary brackish ponds. We found that the eggs –laid in plant shoots during late spring– remained at an early development stage during summer, autumn and winter. This likely increases their resistance to extreme temperatures and illustrates the adaptation of *L. macrostigma* to temporary habitats with uncertain timing and duration of flooding. Yet, embedment in the ice of the plant shoots decreases egg survival, suggesting that higher water levels above the shoots would reduce egg mortality caused by lower winter temperatures. Hatching lasted from mid-March to mid-April and was synchronised. Differences in hatching phenology among ponds were most probably due to differences in water temperatures.

Water salinity is a major driver of insect communities. Hence, we studied in the laboratory the effect of salinity levels experienced during the egg and larval stages. We found no effect of sea salt on egg survival (hatching success) in the range 2–9.5 g/L. By contrast, larval survival increased with salinity level from 0.5 to 8 g/L but was reduced to zero at 16 g/L. Increasing salinity levels augmented



development time until the adult stage, decreased larval growth rates and reduced adult size at emergence. The salinity level experienced at the larval stage had no effect on adult thorax muscle mass, flight performance, investment in immune function and available energy. However, energy consumption increased with higher salinity levels, resulting in a lower net energy budget in the adult stage. Our results therefore indicated that *L. macrostigma* is a saline generalist (fundamental niche) of which the realised niche shifted toward brackish waters.

Other factors, especially biotic interactions, experienced during the larval stage may have consequences on larvae and carry over to affect the adults. We therefore led a field study across 33 ponds, testing for the effect of 14 biotic and abiotic variables on *L. macrostigma* larval density and adult size at emergence. We found that larvae were more abundant in smaller ponds and with later flooding in autumn–winter. This seemed mediated by predatory aeshnid dragonfly larvae, which were more abundant and bigger in earlier flooded ponds. A high susceptibility of *L. macrostigma* larvae to predators could be related to their high activity levels associated with their fast growth and development rate imposed by the drying out of their temporary habitats. Further, *L. macrostigma* tended to emerge larger from ponds with higher water levels, most probably because the lower water temperatures extend the larval growth period. By contrast, we found no effects of water salinity levels and availability of oviposition plants on *L. macrostigma* larval density.

In order to investigate whether the preference adults have for certain plants to lay their eggs benefit the progeny, we used semi-controlled conditions to study the effect on hatching success according to the flooding date and the type of plant shoot where the eggs were laid. We found that wetting was mandatory for hatching. Eggs were able to cope with desiccation until flooding as late as April (i.e. after 10 months in unflooded conditions) –yet with low survival rate– but later flooding resulted in no hatching. Laying eggs in the Sea club-rush *Bolboschoenus maritimus* provided higher survival rate and faster embryonic development. Given that *B. maritimus* grows in deeper parts of the pond, thus experiencing earlier flooding, this suggests that the preference of adults for this plant is an adaptive response to the uncertain flooding conditions of *L. macrostigma* typical habitat.

The results of these studies, which integrated key stages of the life cycle of *L. macrostigma*, provide a scientific basis to develop conservation actions targeting not only the species, but also the restoration of Mediterranean temporary brackish ponds when using *L. macrostigma* as flagship species. Managing a hydroperiod from November to June is crucial in reducing egg desiccation risks and in favouring its larvae over that of other odonate species in the study region. This may require ecological engineering at the watershed scale, based on modelling the pond hydrology using meteorological and topographic data. Given climate change and the increase in the unpredictability of

rainfall in the Mediterranean area, flooding some ponds artificially by irrigation may guarantee the sustainability of a number of perennial reproduction sites. Irrigation may also help control salinity levels and maintain high water levels during the flooding period in order to increase the species' fitness. The colonisation of newly created ponds by *B. maritimus* should be accelerated by transplanting tubers and ramets collected in situ. The existing vegetation cover should be maintained by implementing an adaptive grazing scheme by livestock linked to the target hydroregime. Considering the past degradation of temporary brackish ponds, a network of these habitats should be restored or recreated using *L. macrostigma* as a flagship species. Their long-term monitoring would help identifying the reasons of the project's success or failure, and improve the design of future projects. In this regard, new research about reintroducing *L. macrostigma* in some restored ponds is necessary. This PhD provides an important case study about how to take into account threatened insects within biodiversity conservation strategies.

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## General introduction

### *Impact of human activities on biodiversity and conservation policies*

Increasing human activities are associated with various perturbations of the environment, such as land transformation, fragmentation of natural habitats, chemical pollution, intensive agriculture, spread of invasive species, over-exploitation of resources, and production of wastes (Sponsel 2001). The extent, intensity and rate of these effects are so severe that there is consensual evidences for a sixth mass extinction of biodiversity (Barnovski et al. 2011; Pievani 2014). Since the beginning of the 20<sup>th</sup> century, the scientific community has repeatedly called for biodiversity protection, as does the last Living Planet Report which highlights a decrease of vertebrate populations by 68% over the last 50 years (WWF 2020). The public awareness of the value of biodiversity and ecosystems' services (e.g. Costanza et al. 2014) has gradually increased and resulted in various global policies for the conservation of nature (Mésleard & Alard 2014). In 2010, the states participating to the Nagoya conference on the Convention on Biological Biodiversity committed to reduce the biodiversity loss rate by at least 50%, and to restore at least 15% of degraded habitats by the next decade.

However, stakeholders fail in achieving the goals defined by the successive policies and biodiversity loss is still ongoing, and at best slowed down (Bolam et al. 2020). One of the reasons is that economic growth is, on the one hand, coupled to biodiversity loss and, on the other hand, still advocated in conservation policies (Otero et al. 2020). For example, the last reform of the European Union common agricultural policy 2014–2020 did not take seriously biodiversity conservation into account (Pe'er et al. 2014). With economic growth maintained as paradigm, the recently emerged 'no-net-loss' approach translates into biodiversity offsets, in order to balance for new development projects. However, this approach allows for new habitats' destruction with uncertain gain for diversity, often with under-evaluation of natural ecosystem value, resulting in true loss of biodiversity (Bull et al. 2013; Moreno-Mateos et al. 2015a, Guillet & Semal 2018; Weissgerber et al. 2019). In this context, ecosystem restoration emerges as the paradigm for the next decade 2021–2030 according to the United Nations (<https://www.decadeonrestoration.org/>). This paradigm has already been transferred to the European level (European Commission 2020).

Ecological restoration consists in assisting intentionally the recovery of an ecosystem that has been degraded, damaged or destroyed by any human activity (Clewett et al. 2004). Ecological restoration is needed for biological conservation purposes when the degradation has surpassed an irreversibility threshold, i.e. a natural resilience of the ecosystem cannot occur (Whiteland 1999; Hobbs & Norton 2004). Restoration ecology, i.e. the scientific discipline which ecological restoration relies on, has been formalized by conservationists in the late 80s (Dobson et al. 1997; Dutoit et al. 2014). One should refer to ecological restoration *sensu stricto* only when the operation aims at recovering the ecosystem's species diversity, communities' structure and ecological functioning before being degraded. Yet, we have learnt that 100% successful habitat restoration (i.e. recovering all features of the original habitat) is unrealistic (e.g. Hilderbrand et al. 2005). Hence, restoration most often cannot meet the fundamental requirements of biodiversity offsets *per se* when habitats of high conservation value are impacted, and rather results in mitigation measures (Schoukens & Cliquet 2016). Instead, restoration often takes the form of 'habitat rehabilitation', i.e. restoring some functions of the original habitat. Ecological restoration *sensu lato* refers to 'habitat reassignment' (targeting an ecosystem clearly different from that before degradation) and 'habitat (re)creation' i.e. (re)creating a habitat using ecological engineering. A challenge for conservationists restoring an ecosystem *sensu stricto* remains to approach as close as possible the characteristics of a target ecosystem. This target ecosystem, rather than being an original 'reference' ecosystem, should be an ecologically viable ecosystem, i.e. that was little transformed by human activities or that was assessed as having a favourable conservation status while integrating a history of human activities, and that takes into account the global landscape, the socio-economic context and future environmental changes such as global warming (Aronson et al. 1993; Prach et al. 2007; Jackson & Hobbs 2009). This implies the need to have a thorough knowledge of such target ecosystem in order to accurately design restoration programs.

### *Mediterranean temporary brackish ponds*

#### *Temporary waterbodies*

Freshwaters are biodiversity hotspots as they support ca. 10% of species while occupying less than 3% of the world's surface (Gardner & Finlayson 2018). Freshwaters also support human activities, and they represent valuable natural resources, in economic, cultural, aesthetic, scientific and educational terms (Dudgeon et al. 2006; Costanza et al. 2014; Mitsch et al. 2015). On another hand, freshwaters are subject not only to the threats affecting terrestrial and marine habitats (see *Impact of human activities on biodiversity and conservation policies*), but also to flow and regime modification (e.g. Custodio 2010;

Hall et al. 2014). Additional threats have also recently emerged such as microplastic and nanomaterial pollution, infectious diseases, salinization and calcium decline (Reid et al. 2018). The increase in the extent of wetland area reported in some literature is actually due to an improvement of mapping technologies, natural wetland loss and conversion actually following a continuous increasing trend, especially in the Mediterranean (Beltrame et al. 2015; Davidson et al. 2018). Another confusing phenomenon is the increase in the total area of open waters in the Mediterranean, actually due to dam construction and therefore to the creation of big reservoirs, of which the negative ecological impacts are well known (Perennou et al. 2020).

The conservation of freshwater species is especially worrying because of their susceptibility to climate change, with often higher extinction rates than those of terrestrial taxa (Heino et al. 2009). For example, freshwater vertebrate populations decreased by 84% over the last 50 years, i.e. more than the 68% assessed for all vertebrates (WWF 2020). In the context of global warming, the future of temporary waterbodies and their biodiversity is especially worrying. The conservation value of temporary waterbodies and of ponds, and in combination of Mediterranean temporary ponds (MTPs), is well established but they have received scientific interest only recently (Bagella et al. 2016; Hill et al. 2021; Bagella 2023). However, their destruction has considerably accelerated at the end of the 20<sup>th</sup> century (Zacharias & Zamparas 2010). This makes freshwaters and MTPs top-priority ecosystems that conservation efforts must target (Dudgeon et al. 2006), and the restoration or (re)creation of temporary waterbodies are considered key strategies in this context (Zedler et al. 1993; Tickner et al. 2020). Such is especially relevant in coastal regions as the majority of them were degraded (Williams et al. 2021).

### *Hydrological functioning of temporary brackish waterbodies*

The water regime of temporary waterbodies is singular: they experience a recurrent dry period of varying timing (when?) and duration (how long?), this water deficit period being due to a reduced amount of precipitations (either directly or through their watershed) and an excessive evaporation (Williams 1987; Fig. I.1). This definition of temporary waterbodies is broad and can refer to very different habitats depending on whether water is standing (temporary ponds) or running (temporary streams), and on whether flooding occurs several times a year, once a year with a seasonal cycle or after several years but regularly (Brock & Casanova 1997). For example, snow melting produces temporary waterbodies with a relative predictability. Contrastingly, in arid or semi-arid conditions, flooding depends on unpredictable climatic events. Under the Mediterranean climate, temporary ponds have an annual cycle with a flooded period during typically wet and mild winter and a drought



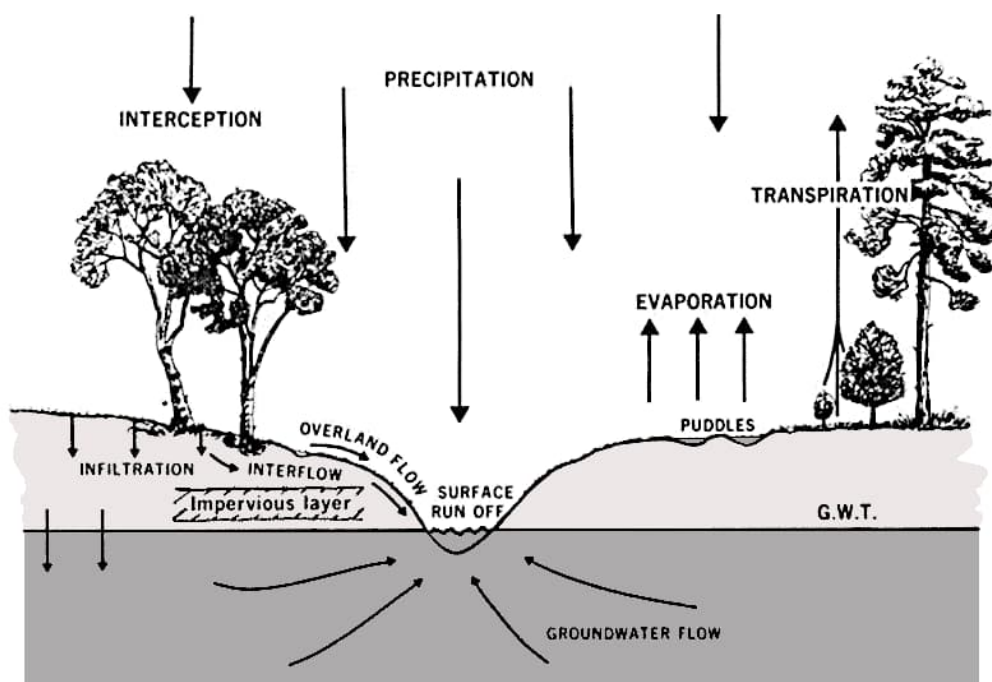


Figure I.1 – Runoff components that contribute to the water of a pond or stream; GWT refers to ‘ground water table’ (after Williams 1987).

period during hot and dry summer (Dimitriou et al. 2009). The flooding and drying processes of a waterbody will respectively dilute and concentrate the dissolved salts. Depending on climate conditions (i.e. evaporation vs rainfall amount and timing), the salinity level of MTPs may vary both in mean and in range across and between years, as the hydroperiod can vary in duration and water levels during the flooded period can fluctuate.

Hydroregime (including hydroperiod, i.e. the timing and duration of the flooding period) and salinity have been well identified as critical drivers of plant communities (e.g. Grillas 1990; Grillas & Battedou 1998). Hence, MTPs regroup different types of habitats along a salinity gradient interacting with flooding duration. Their salinity level ranges from 0.5 to 40 g/L (Aguesse 1957) but those salted from ca. 5 to 40 g/L have been overlooked despite their conservation value (e.g. Morgan 1982; Chentoufi & Rokneddine 2004). A large part of the literature dealing with the conservation of MTPs considered actually fresh waters (e.g. Bagella et al. 2010; Rhazi et al. 2012). Zacharias et al. (2007) proposed a typological classification of MTPs based on their environmental conditions but all waters with salinity above 3 g/L were pooled in the same group. MTPs are considered as priority habitats according to the Habitats Directive (European Council 1992) but this habitat (code ‘3170’) actually regroups only four subtypes of fresh to slightly salted waters. Coastal lagoons are also considered by the Habitats Directive (code ‘1150’) but they are far more salted, and most often permanent. Hence, conservation efforts likely overlook MTPs of a large range of salinity levels.

### *Study area*

All studies presented in this PhD thesis were made in southern France, and more precisely in the Camargue i.e. the River Rhône delta (Fig. I.2). This region belongs to the Mediterranean basin, which comprises the lands that surround the Mediterranean Sea and that have a Mediterranean climate. It stretches ca. 3,800 km east to west from Portugal to Lebanon and ca. 1,000 km north to south from Italy to Morocco and Libya (Sundseth 2009).

### *Mediterranean climate*

The Mediterranean climate is characterized by temperatures mild to cool in winter and warm to hot in summer, during which a water deficit occurs (Fig. I.3a). In the Camargue, this water deficit is increased by the strong dry winds coming from the north and the east, the Mistral and the Tramontane (Fig. I.3b). Because of the interaction of day length, temperatures and winds, evaporation is highest in spring and summer, with an annual mean of 1458 mm (SD = 72 mm, period 1994–2023, Météo France, meteorological station n°13004003 of 'Arles-Valat', 43°30'36"N | 4°41'38"E). This results in a drought season, generally from late spring to early autumn, during which MTPs are unflooded. Yet, rainfall is highly variable: for example, during the period 1994–2023, annual rainfall was 551 mm  $\pm$  187 mm (mean  $\pm$  SD, Météo France®), most of which occurred in autumn (38%) and in winter (22%). Given the irregularity of autumn rainfall, the flooding of MTPs is typically poorly predictable and can occur between September and January, but most of the time in late autumn.

### *Camargue's geomorphology*

Today's geomorphology of the Rhône delta is the heritage of the progress to the south of fluvial sediments over the last ca. 7,000 years, despite regular marine intrusions (Arnaud-Fassetta et al. 2005). The past presence of the sea in the actual southern part of the delta results in a salinity gradient in the sediment, and in the confined aquifer from ca. 2 g/L in the northern part of the delta to ca. 43 g/L in its southern part (de Montety et al. 2008). Further, fresh water incomes (e.g. from rainfall) flush the salt from the level of the soil deeper into the sediment. Hence, hydrology and salinity show opposite trends along the topographic gradient, which drive the occurrence of the different plant species given their specific salinity tolerance, and therefore the Camargue's habitats. Yet, this pattern is strongly modified by human activities with desalinization in the northern part of the delta (for agriculture) and enhanced salinity in the south (in saltpans). Human activities have had a significant impact on the Camargue habitats, especially since the containment of the River Rhône and the Mediterranean Sea in

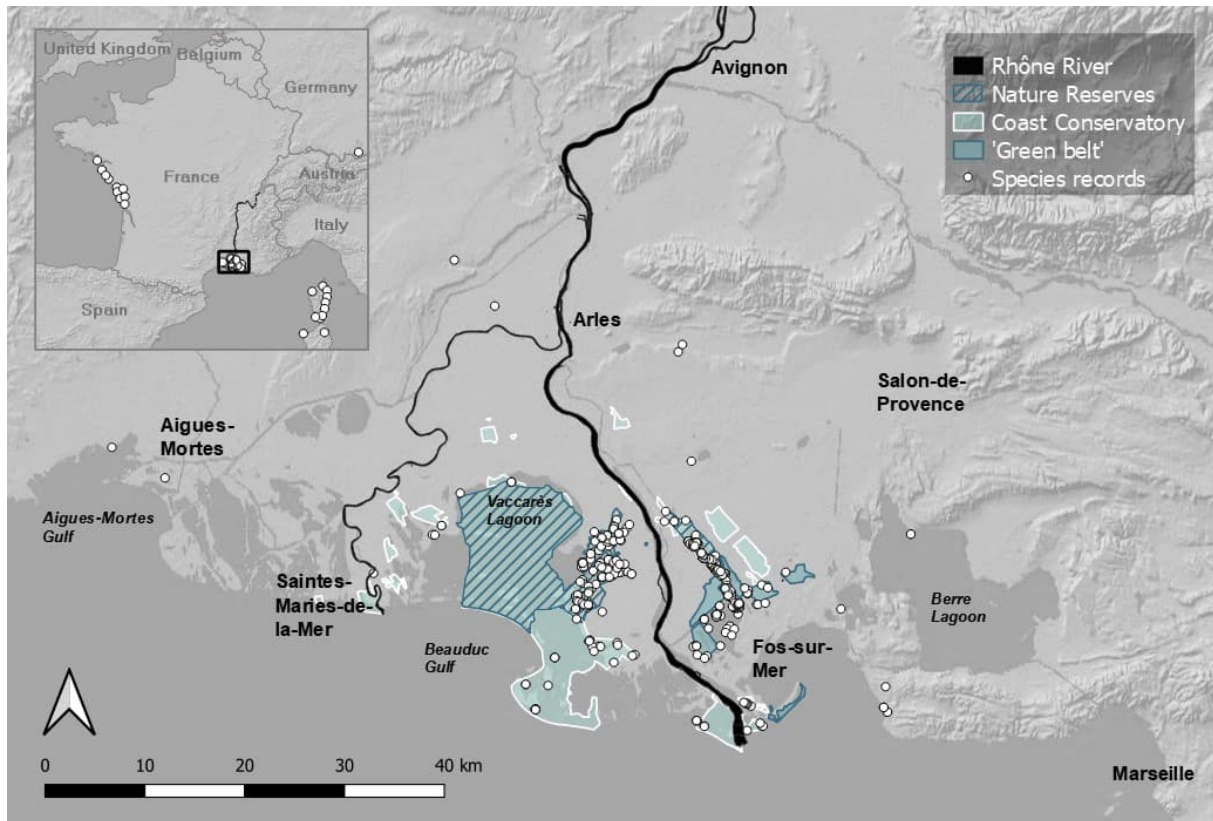


Figure I.2 – Location of the Camargue in southern France (black rectangle within the upper left panel), with an overview of the observation sites of the damselfly *Lestes macrostigma* (white dots). The River Rhône takes its source in Switzerland, flows southward, and splits at Arles city into the Smaller Rhône (flowing till the Saintes-Marie-de-la-Mer city) and the Greater Rhône (flowing till Fos-sur-Mer city). The Camargue, i.e. the River Rhône's delta, geomorphologically extends from the gulf of Aigues-Mortes city to the gulf of Fos-sur-Mer, and from Arles city to the Mediterranean Sea. Several natural habitats benefit of various protection status such as Nature Reserves, sites of the French Coast Conservatory, or the 'green ring' of Marseille-Fos industrial port (source: ESRI Shaded Relief © | projection: WGS84 | odonatological data: literature and databases review | realisation: P. Lambret).

the middle of the 19<sup>th</sup> century. River floods and marine intrusions could not occur anymore, thereby stopping the natural hydrodynamic functioning of the delta. Except through rainfall, the flooding of wetlands is now under control, using an irrigation network made of pumping stations, dikes and ditches (Chauvelon et al. 2013).

*Local natural heritage and conservation issues*

The Mediterranean basin is a biodiversity hotspot in Europe (Cuttelod et al. 2008), with particularly the Camargue having a high biodiversity: its conservation value has been well established by several national and international programs (Natura 2000, site of the Ramsar Convention on Wetlands, reserve of the 'Man And Biosphere' UNESCO program), and several areas benefit a legal protection such as the nature reserves (Fig. I.2). Nevertheless, the Mediterranean basin is experiencing a tremendous anthropogenic impact (Cramer et al. 2018) and future modelling scenarios predict that Mediterranean biomes will experience the greatest change in biodiversity, due to the influence of land use and climate change (Sala et al. 2010). As in other regions, biodiversity in the Camargue is declining, especially in amphibians, odonates and orthopterans, reflecting the relative failure of protection measures mainly against agricultural influences (Fraixedas et al. 2019). Since the early 1940s, land modifications in the Camargue have resulted in the net loss of 40,000 ha of natural habitats, including 33,000 ha of wetlands (Tamisier & Grillas 1994), their coverage decreasing from 67% in 1942 to 39% in 1984 (Fig. I.4). More recently this percentage reincreased, especially with the land acquisition of former saltpans by the French Coast Conservatory. From the late 19<sup>th</sup> century, these modifications consisted in the development of the irrigation network, the division of marshes into smaller dyked units and their levelling. This allowed shifts in land-use (agriculture, salt industry, waterfowl gaming, wildlife conservation), some of which ask for artificial water regimes mainly consisting in large freshwater inputs, sometimes opposite to the natural cycle (e.g. rice fields with a dry period in winter and a flooding period in summer). In the 1970s, the French government developed over the marshes of Fos-sur-Mer area an industrial port complex, which is still growing nowadays. Agriculture and industry are therefore responsible for the huge increase of artificial and fixed hydrological functioning, decreases of the mean salinity of habitats, and decreases of natural habitats, including temporary brackish waters. This motivates the need of restoring these habitats. Yet, the often multitude of land users, and their historical succession, provide a complex social canvas that conservation and wildlife managers have to take into account for a better efficiency (Mathevet et al. 2015). The success of restoration programs may thereby rely on bridging ecological, social and economic matters (Biwas et al. 2009). In this context, it may be helpful to use a flagship species, i.e. a charismatic species of which conservation will benefit a whole ecosystem and anchor a conservation program (Simberloff 1998; see *Using Lestes macrostigma for the conservation of temporary brackish ponds*).

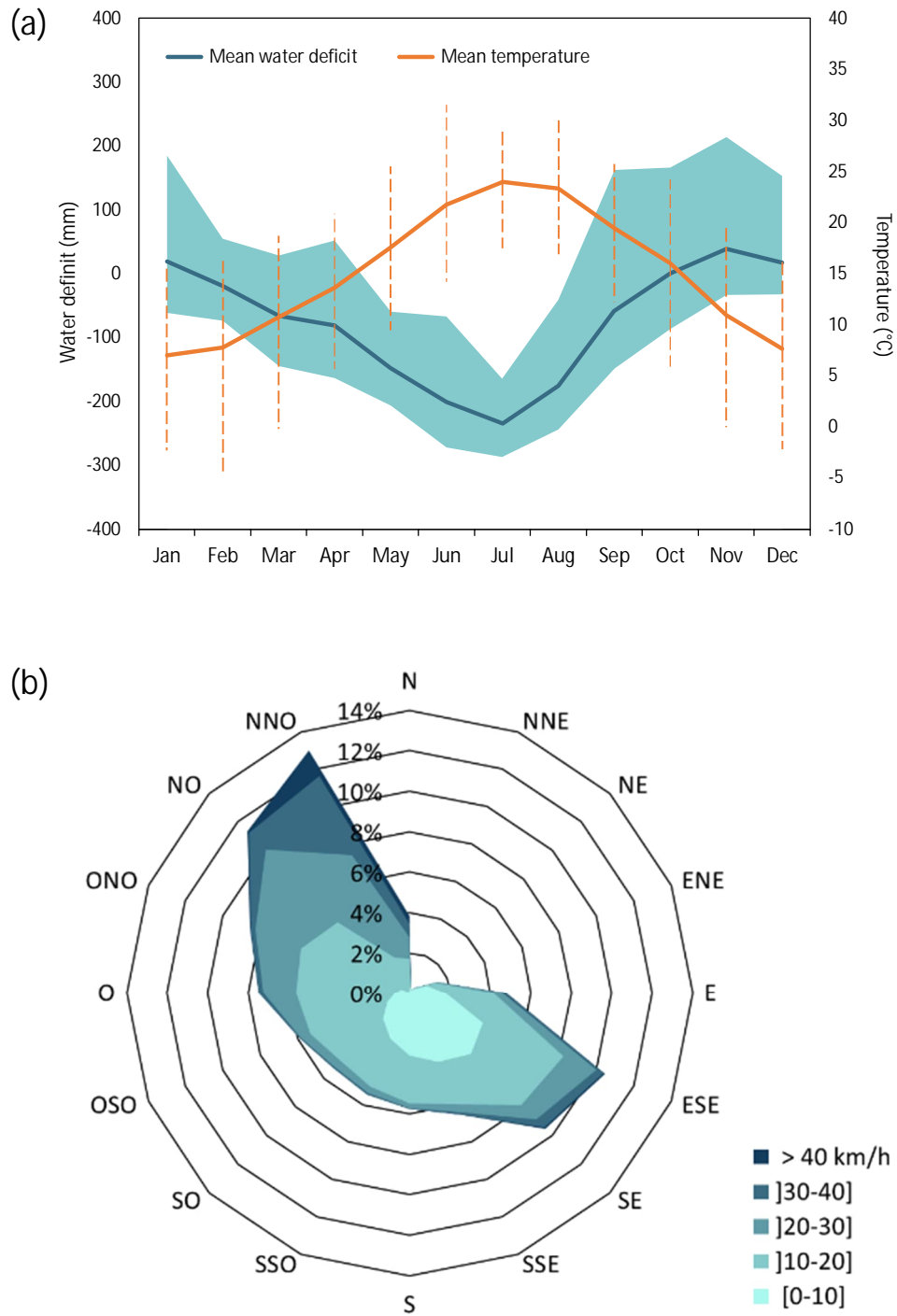


Figure I.3 – Characteristics of the Mediterranean climate at Tour du Valat research institute during the period 1994–2023: water deficit (i.e. difference between rainfall and evapotranspiration) and air temperature (dotted lines indicate monthly minima and maxima) (a) and wind rose (b) (data: © Météo France).

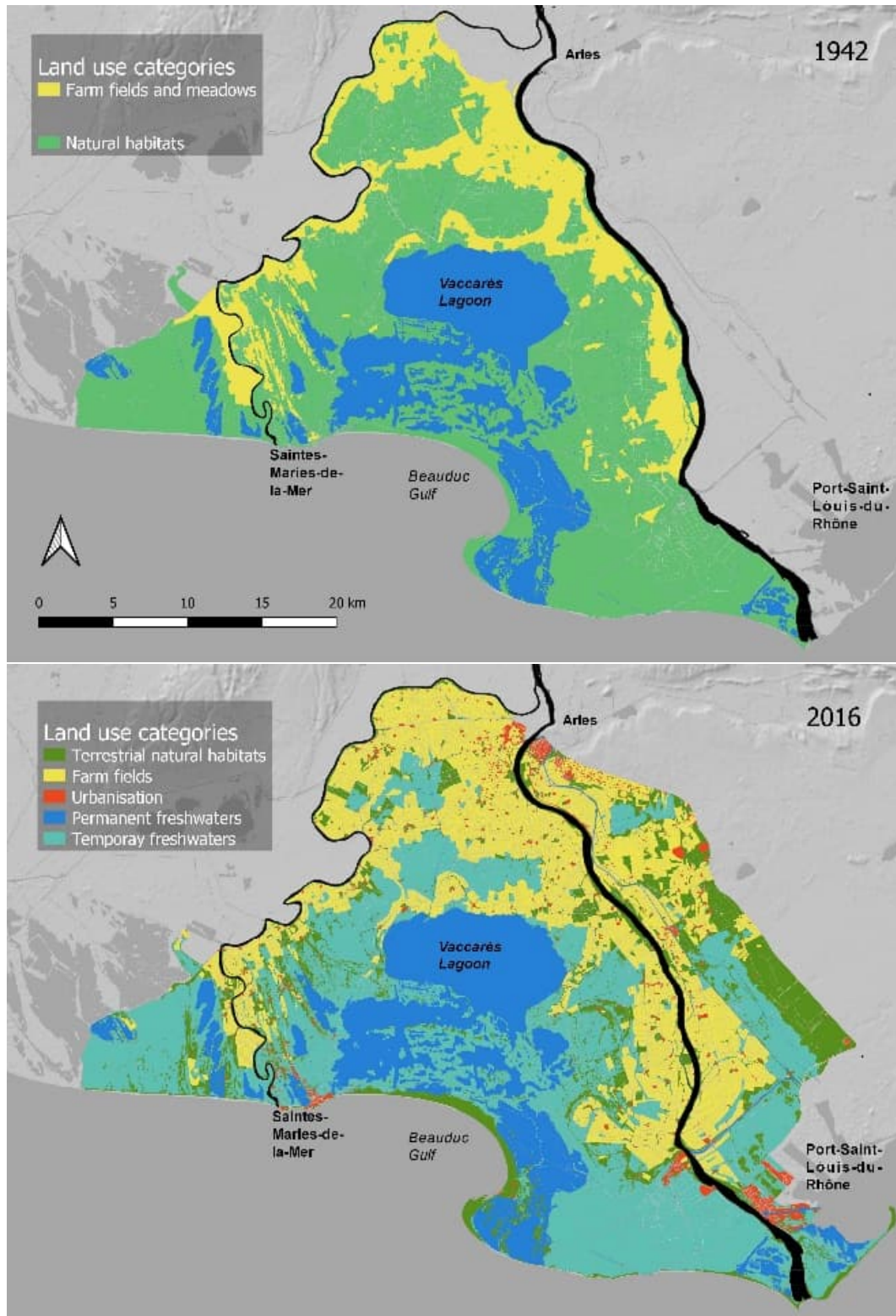


Figure I.4 – Reduction of the cover of natural habitats in the Camargue between 1942 and 2016 (source: ESRI Shaded Relief ©, projection: WGS84 | data: National Society for Nature Protection – Camargue National Nature Reserve ©, Camargue Regional Natural Park © | realisation: P. Lambret).



### *Hydroregime and salinity as drivers of animal communities*

The occurrence of a dry phase in the water regime of water bodies is a strong environmental constraint. The ephemeral character of the aquatic phase acts as a first level filter because it selects the aquatic taxa trying to establish populations in such habitat. Further, the characteristics of the hydroregime (depth, timing, duration, frequency, and predictability of water) shape invertebrate communities (Sim et al. 2013). For instance, the taxonomic composition of insect communities shifts across the hydroperiod gradient and water level fluctuations, with the different communities containing unique taxa (Whiles & Goldowitz 2005; Jooste et al. 2020). There are no aquatic vertebrates that are permanent residents able to cope with this periodical constraint, except amphibians and some rare fishes (Williams 1987; Van der Broeck et al. 2015). Instead, animal communities are composed of ‘invertebrates’ and dominated by arthropods species, which show diverse adaptations to cope with this hydroperiod constraint (Williams 1987, 1996; Fig. I.5). Two general strategies can be identified: (1) adults of aquatic (e.g. boatmen and diving beetles) and semi-aquatic (e.g. mosquitoes and odonates) species can fly to emigrate from the drying environment and are hence called dispersers, or temporary inhabitants; or (2) individuals of aquatic species (e.g. water fleas, scuds) can enter a resistant life-history stage or form, and are hence called residents or permanent inhabitants. The composition of temporary waterbody communities therefore relies on colonisation in space (by dispersers from outside the waterbody) and in time (by hatching phenology of residents within the waterbody which differs across taxa).

Yet, this classification as resident or dispersers may be tricky for some taxa during their larval life-stage. Indeed, unlike crustaceans that have both aquatic larvae and adults, e.g. anura (frogs and toads), dipterans (e.g. mosquitoes and chironomids, horse flies) and odonates (damselflies and dragonflies) have a complex life cycle with strictly aquatic larvae and terrestrial or amphibian adults. While adults are able to disperse far away from the larval habitat, larvae are ‘resident’ in that they cannot escape unfavourable conditions. Further, all insect larvae are wingless and only few of them are to enter a resistance stage (Strachan et al. 2015). Temporary waterbody insects therefore face the challenge to achieve their larval development, i.e. to reach the ‘disperser stage’, before the habitat dries out. To do so, they usually show larval peculiar life-history traits such as a rapid growth and development (Williams 1987; Strachan et al. 2015). In an uncertain environment, the timing of egg hatching is therefore a critical step. One bet-hedging strategy is germ banking by spreading hatching across and within flooding events in order to provide hatchlings with various environmental conditions and to reduce intraspecific competition (Danforth 1999; Evans & Dennehy 2005). Yet, triggering the hatching of most of eggs with various environmental cues may synchronise hatchlings with some resource opportunity (Williams 1998).

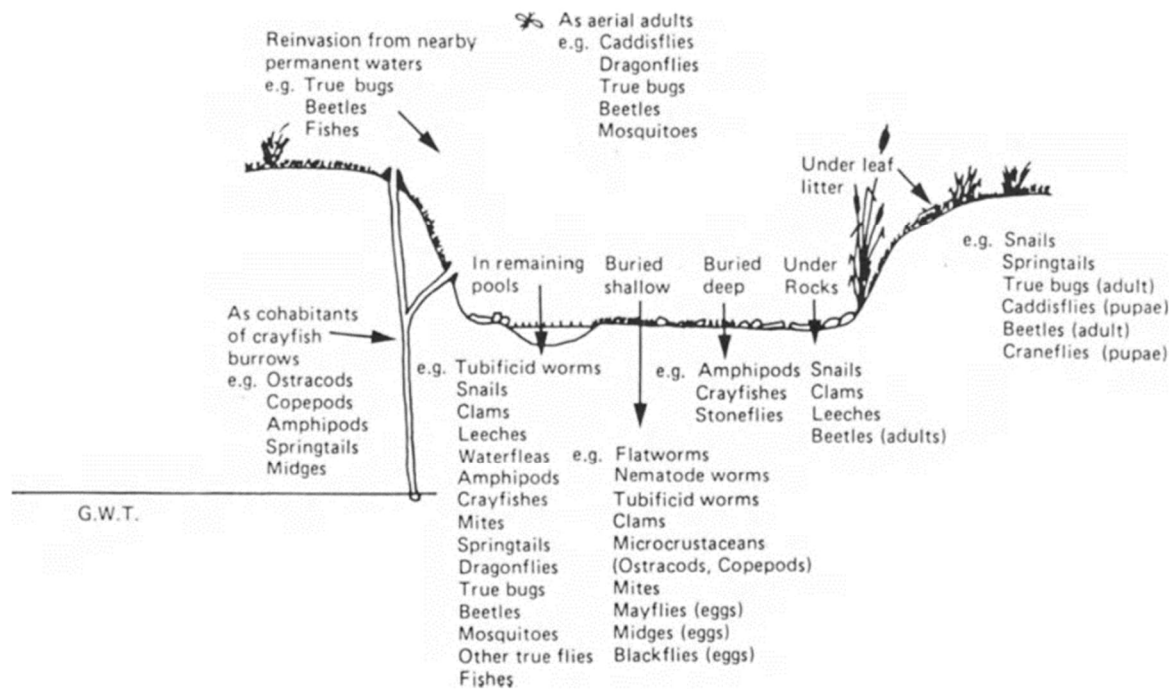


Figure I.5 – Different strategies to cope with the dry phase of temporary ponds (Williams 1987).

The water regime of a temporary waterbody characterizes its aquatic phase with its duration (e.g. few days to several months), its frequency (e.g. once a year or once a decade in average), its timing (e.g. autumn–winter for MTPs, spring in the case of the artificial flooding of rice fields) and its variation of water level across time (Brock & Casanova 1997). Hence, the hydroperiod (i.e. the timing and the duration of the flooding period) of temporary waterbodies that are naturally flooded, by either rainfall and/or river rises, will depend on the timing and amount of local rainfall and/or rainfall over the watershed (Timms 2012). The hydroperiod is therefore likely to vary from one year to another, especially in the Mediterranean region (see § *Study area*). This timing will interact with other factors varying in time such as temperature, photoperiod, and the phenology of communities outside the waterbody. Such temporal aspects will act as a second level filter for species living in temporary waterbodies (Florencio et al. 2009). Populations of a temporary waterbody, although established, are susceptible to unfavourable conditions affecting the species reproductive success. For instance, Waterkeyn et al. (2011) showed that pluri-annual persistence of unfavourable conditions in a temporary waterbody leads to shifts in the living crustacean communities but also of the resting egg bank, possibly leading to the extinction of unfavoured species. Further, water environmental factors (e.g. temperature, pH, salinity, chlorophyll a) will vary along flooding and drying events during one year. These hydrological variations within a year lead to the succession of several animal communities (Boix et al. 2004).



Flooding and drying events translate into dissolution and concentration of the dissolved solids (i.e. ions, or salts), so that water salinity can temporarily reach high levels (see § *Freshwaters and Mediterranean temporary brackish ponds*). Increasing salinity is another filter to the communities of the temporary waterbodies. These two factors, hydroperiod and salinity, are well-known drivers of animal communities. For instance, Whiles & Goldowitz (2005) showed along a gradient from ephemeral to permanently flooded waterbodies that mean annual macroinvertebrate abundance and biomass increased with hydroperiod duration, but that taxon richness and diversity peaked in temporary sites, together with the evenness of distribution of functional groups. Under both cold-temperate and Mediterranean climates, Brucet et al. (2012) showed that richness and abundance of most plant-associated macroinvertebrate taxa decrease with salinity, probably in relation to the plant biomass (Grillas et al. 1993). Waterkeyn et al. (2008) further showed in the Camargue that increased duration of the hydroperiod has a positive effect on crustacean species richness, while increased salinity has the reversed effect; moreover these two factors interacted as salinity only caused a lower richness in ponds with a longer hydroperiod. The interaction of hydroperiod and salinity was also shown across different wetlands in China where the abundance and richness of snails were higher in some permanent waterbodies than in temporary waterbodies, and higher in some freshwaters than in saline-alkaline waters (Wu et al. 2019). However, in this study, the salinity gradient was dominant over the hydroperiod gradient, and salinity mediated the impacts of hydroperiod: drying events had negative effects in freshwater habitats while perennial flooding intensified the exposure to salt in saline habitats. That salinity may be a stronger driver than hydroperiod in shaping animal communities was also suggested by Boix et al. (2008) when studying 91 waterbodies which were distributed along hydroperiod and salinity gradients in Catalunya (north-eastern Spain, Mediterranean climate). They found that the crustacean and insect communities were similar in temporary and permanent saline waters, but differed from the communities of permanent freshwaters on the one hand, and of temporary freshwaters on the other hand.

Shifts in odonate communities were also shown along hydroperiod gradients. For instance, Stoks & McPeck (2003a, 2003b) studied the communities of spreadwings (Zygoptera: Lestidae) in New England (USA) across a gradient of hydroperiod and predator presence. They found that *Lestes* spp. segregate along the hydroperiod gradient based on their life-history traits, some spp. being unable to cope with dry periods; and that some species had no efficient anti-predator responses against large aeshnid dragonfly larvae (Anisoptera: Aeshnidae) and fishes, thereby explaining their restriction to predator-free waterbodies. Odonate community shifts have been more rarely studied along a salinity gradient (but see Zia et al. 2018). In a rare example study, Uboni et al. (2020) showed that across coastal waterbodies of the northern Adriatic coastline (Italy) the species richness of odonates was

higher in freshwater and oligohaline waters than in polyhaline waters. An even more relevant work regarding the impact of both hydroperiod and salinity on odonate communities was made in the Camargue, in the frame of the PhD thesis of Aguesse (1961). He notably showed that the communities of rice fields, which are temporary waterbodies showing a reversed hydroperiod (drought in winter and flooding in summer), depended on the colonisation from other types of waterbodies, by species showing multivoltinism (i.e. several generation per year) as one of their life-history traits. He also showed that species richness decreased with increasing salinity: while *Lestes barbarus*, *L. sponsa*, *Anax parthenope* and *Sympetrum fonscolombii* can reproduce successfully in brackish waters, *L. macrostigma* and *Ischnura elegans* are the only two species able to cope with salinities over 16 g/L. Further, the timing and the duration of the hydroperiod appeared to be especially responsible for the inter-annual variation of the abundance of species reproducing in temporary wetlands. The hydroperiod may also be associated with other environmental factors, such as vegetation. For instance, Carchini et al. (2007) found that the number of odonate species increased with the duration of the hydroperiod and the species richness of macrophytes. Such biological association (macrophytes and odonates) may rely on the same physical factors driving both communities. Indeed, hydroperiod and salinity are also well known drivers of plant communities (e.g. Grillas 1990; Grillas & Battedou 1998; see also § *Freshwaters and Mediterranean temporary brackish ponds*).

### *Using Lestes macrostigma for the conservation of temporary brackish ponds*

Using the protection status of species in identifying conservation issues may be misleading (Cassidy et al. 2001). Instead, conservationists identify the importance of species mostly with regard to their role in the ecosystem (see Simberloff 1998). Species can be labelled as (1) 'target', (2) 'keystone', (3) 'indicator', (4) 'umbrella' and/or (5) 'flagship' species, each referring to a different concept. (1) A target species is a species to which conservation efforts are directed, due to its protection status (regarding the Habitats Directive) or extinction risk (regarding the red list of the International Union for Conservation of Nature [IUCN]). (2) A keystone species can be defined as a species whose removal from the food web would lead to a reduction in the number of species and an associated strong change in the community structure (Cottee-Jones & Whittaker 2012). (3) The absence/presence of an indicator species at a site testifies of a deterioration of the ecological integrity of this site (Carignan & Villard 2002). (4) An umbrella species is characteristic for a habitat and has a sufficient large range of habitat subsets so that its conservation benefits many other species occupying subsets of this habitat. (5) A flagship species is a charismatic species –usually a large mammal or bird– of which conservation will benefit a whole ecosystem and that can therefore be used to anchor a conservation program among

various stakeholders. Yet, not all charismatic species are at the same time umbrella species and some of these therefore deserve no priority conservation interest (Caro et al. 2004). Instead, a less charismatic species, such as an insect, may serve as flagship more efficiently (Home et al. 2009). Seddon & Leech (2008) emphasized that conservation managers applying the umbrella species concept should especially focus on seven criteria: a well-known biology, a large home range size, a high probability of population persistence, the co-occurrence of species of conservation interest, some management needs that are beneficial to co-occurring species, a sensitivity to human disturbance, and an ease of monitoring. Note that these recommendations appears to be relevant for the species belonging to the other concepts too.

Most research evaluating the conservation value of temporary ponds focused on plant species, as indicated in the EU Habitats Directive (e.g. Pinto-Cruz et al. 2009; Rhazi et al. 2012). Unfortunately, plants are poor flagship candidates (Pany & Heidinger 2017). The numerous crustaceans inhabiting brackish to saline temporary water received so far little attention despite their conservation interest (Marrone et al. 2006). Amphibians could serve as flagship species but they are usually restricted to low levels of salinity (e.g. Bagella et al. 2010) and therefore cannot be used for the conservation of temporary brackish waters. Instead, some insects may be good flagship candidates that occur also in temporary brackish ponds. As all insects, freshwater insects are facing a worldwide decline, especially due to agriculture intensification and use of pesticides (Sánchez-Bayo & Wyckhuys 2019). In freshwaters, more than 25% of 'invertebrates' are threatened (Macadam & Stockan 2015). Despite the key role freshwater 'invertebrates', including insects, may play in the ecosystems, they have been overlooked and data are missing to assess their conservation status (Gozlan et al. 2019). The discrepancy between (1) the importance of insects in ecosystem functioning compared to vertebrates and (2) the conservation efforts each group receives has been established a long time ago (Miller 1993). The strong decline of insects not only severely threatens the functioning of ecosystems, but also the services they provide to human populations such as food resource (including through fisheries) and pest control (Chagnon et al. 2014; Macadam & Stockan 2015). Indeed, insects may play a critical role in the functioning of ecosystems, i.e. energy flow and nutrient cycle, especially through the relation herbivorous insects have with plants (Weisser & Siemann 2008). The strength of the impact of species loss on ecosystem functioning depends on their level in the trophic web and the loss of few predators may be as strong as the loss of many plants (Duffy 2003).

In temporary ponds, odonates are among the top predators and therefore may occupy a keystone position in aquatic invertebrate communities (Stoks & McPeck 2003b). They are among the most well-known and extensively studied insects (Corbet 2004; Córdoba-Aguilar 2008) and have been used for decades for the conservation of freshwaters, with stenotopic species being good indicators of

habitat characteristics and habitat changes following restoration actions (Samway et al. 1996). One such stenotopic species is the Dark spreadwing *Lestes macrostigma* (Eversmann, 1836) (Odonata: Lestidae) which is restricted in Europe to temporary brackish waters (Chelmick & Lambret 2020). Other dragonflies may inhabit temporary brackish ponds, such as the congeneric *L. barbarus*, but they cannot occur at as high salinity levels as *L. macrostigma* does (Aguesse 1961). *Lestes macrostigma* can disappear from an area for years (Muñoz & Ferreras-Romero 2011) but this absence does not lead to dramatic community shifts. Hence, *L. macrostigma* unlikely plays a keystone role within temporary brackish ponds. The distribution area of *L. macrostigma* is very patchy but ranges from Portugal to Siberia (Boudot & Raab 2015). According to the criteria of the IUCN, *L. macrostigma* is considered as 'Near Threatened' in the Mediterranean Basin and in Europe, where it was formerly considered as 'Vulnerable' (Riservato et al. 2009; Kalkman et al. 2010; G. de Knijf pers. comm. 2024); it is even threatened at smaller geographic scale, e.g. 'Vulnerable' in Spain, 'Endangered' in France and Italy, 'Critically Endangered' in Bulgaria (Verdú & Galante 2009; Riservato et al. 2014; Popov 2015; UICN France et al. 2016), where this species is therefore a priority target for conservation. Hence, it was proposed to include *L. macrostigma* in the Habitats Directive (Kalkman et al. 2018). Noteworthy, this species already serves as an indicator of favourable conservation status of brackish water habitats (Pont 2014). Given the popularity of dragonflies among insects (Lemelin 2008) and the attention they receive as conservation target or tool (e.g. Hawking & New 2002; Suh & Samways 2005; Clausnitzer et al. 2017; Goertzen & Suhling 2014), *L. macrostigma* is a promising flagship species for the conservation of temporary brackish waters. To meet the criteria that a flagship species should fulfil (see above), particularly the need of a well-known biology needs to be addressed. Yet, several aspects of *L. macrostigma* biology remain poorly understood, as highlighted here after.

### *Study species*

This part uses several of my publications prior to Chapters 1 to 4. The introduction and discussion sections of these former publications largely take into account the existing literature concerning the species. Hence, this part is not a review per se but rather aims at highlighting the knowledge gaps that my PhD thesis filled in.

### Description

*Lestes macrostigma* is the largest species in its genus in Europe. Males and females are very similar: both are metallic black with extensive blue pruinescence<sup>1</sup> and conspicuously large pterostigmata<sup>2</sup> (Dijkstra & Lewington 2006); coloration differences between sexes are slight (Lambret 2013). Eggs are laid endophytically (inserted into plant shoots) and are therefore elongate, approximately 1.4 mm long (Matushkina & Lambret 2011) and 0.3 mm in diameter (Jödicke 1997). The ‘hourglass’ shape of the prementum<sup>3</sup> in *L. macrostigma* is a unique and probably the most important diagnostic feature to identify the larvae of this species (Nielsen 1954). At the post-teneral stage (i.e. after emergence, when the cuticle has not completely hardened but flight is already possible despite clumsy), adults are coloured by an unmistakable and striking metallic blue-black (Fig. I.6). This coloration develops approximately 2 h after the beginning of final ecdysis i.e. when the pharate<sup>4</sup> adult dorsum breaks through the thorax of the larval skin (Lambret 2013). The day following emergence, the adults of both sexes start developing a blue pruinescence over the entire body, with the exception of the five median segments (S3–S7) of the abdomen. When mature, their eyes become cobalt blue in the male, and half teal / half navy blue in the female. These features render the species’ identification unmistakable in the field at any stage in its development. This is important for monitoring the spatio-temporal dynamics of the species, and especially relevant when seeking at establishing the autochthony of one population: identifying the species easily and rapidly at the post-teneral stage (i.e. just after emergence) allows to state that one population is autochthonous without the need of searching for exuviae (i.e. the larval exoskeleton the adult leaves after its emergence moult), which is a fastidious and time-consuming task.

### Distribution range

*Lestes macrostigma* has a very extensive, albeit fragmented, distribution (Fig. I.7). It ranges from Iberia across Europe, Kazakhstan and southern Russia to Siberia, China and Mongolia, extending north as far as the 49<sup>th</sup> degree of latitude (Boudot & Raab 2015). Its distribution in southwest Europe is very restricted and the species is only found in four areas: the southwest Atlantic coast (e.g. Jourdes 2009); Corsica Island and Sardinia (e.g. Hardensen & Leo 2011; Berquier & Andrei-Ruiz 2019); the Camargue

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<sup>1</sup> Supracuticular pigmentation reflecting ultraviolet light (see Corbet 2004)

<sup>2</sup> In some insects, including odonates, group of cells on the distal fore part of the wing which are thickened and coloured, and which serve as inertial regulator during the flight (see Corbet 2004)

<sup>3</sup> In odonates, the labium of larvae has evolved in an extensible and prehensile appendage they use to capture their prey.

<sup>4</sup> Before ecdysis, when new and old cuticles are separated; a pharate adult is, before emergence, still inside the larval skin (see Corbet 2002, 2004)

(e.g. Lambret et al. 2009); and the Iberian Peninsula, inland (e.g. Piñeiro Álvarez 2020) and especially along the southern coast of Andalucía (e.g. Muñoz & Ferreras-Romero 2011). The fragmentation of the distribution of *L. macrostigma* in the eastern part of its range may be partly due to a lower surveying effort and therefore a lack of data. However, the distribution of odonates in Western Europe is well-known (Boudot & Kalkman 2015), and *L. macrostigma* populations are undoubtedly isolated in this area.



Figure I.6 – Emergence of a *Lestes macrostigma* female. At the teneral stage (a), when the abdomen and the wings are not yet fully expanded, the species has no colours that allows distinction from another species. However, few hours after emergence (b), the colour pattern renders the species identification unmistakable (© P. Lambret).

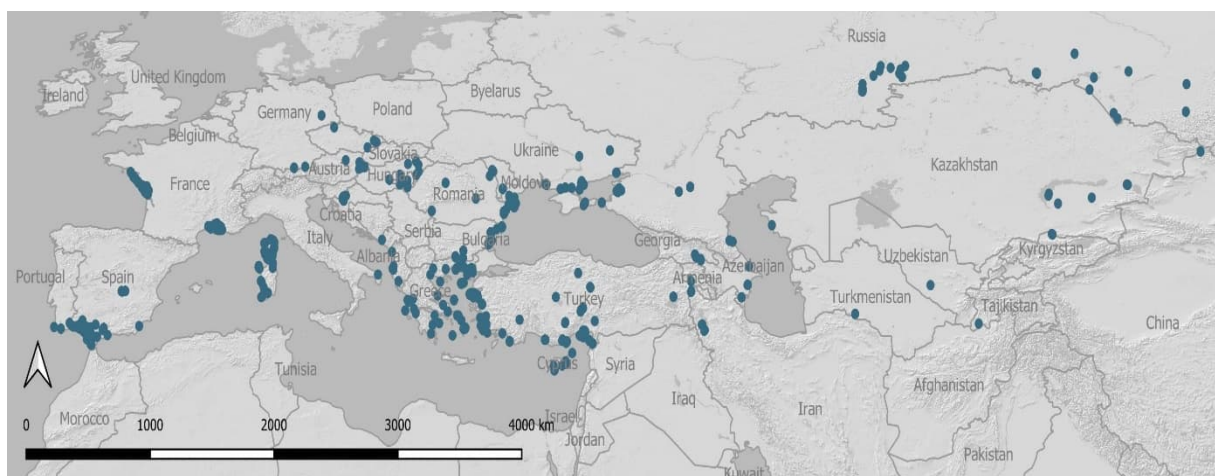


Figure I.7 – World map with observation sites of the damselfly *Lestes macrostigma* (blue dots) showing that the species distribution is fragmented, albeit very extensive (source: ESRI Shaded Relief © | projection: WGS84 | odonatological data: literature and databases review | realisation: P. Lambret).

## Habitat

*Lestes macrostigma* has stenoecious larvae that are most often restricted to temporary, brackish (oligohaline) waters in the western Palearctic (Boudot & Raab 2015; but see Berquier & Andrei-Ruiz 2019), although the species appears to breed more extensively in fresh water in the eastern parts of its range (Kosterin 2015). In the Camargue, successful emergence was recorded from water with salinity values ranging from ca. 0.4 g/L up to ca. 23 g/L (Lambret et al. 2009). Yet, salinity levels usually range from ca. 2.5–4.5 g/L in late March up to ca. 4.5–8 g/L in early May. As detailed information about the effect of salinity on life-history and physiological traits was lacking, this was addressed in Chapter 3. Aguesse (1955) noted that larvae died during the drying phase when the water reached 8.4 g/L and 30 °C. Given that this salinity level is well below the species upper tolerance, this suggests that water temperature above 30 °C is lethal for larvae. Yet, shallow and warm water bodies contribute to its rapid larval development (Schiel & Buchwald 2015a, 2016a). The waterbodies where *L. macrostigma* reproduces can be large temporary marshes (Fig. I.8a) or tiny temporary pools (Fig. I.8b) (Lambret et al. 2009). Larvae show a plant-dwelling behaviour (Fig. I.9): their abundance is more than 20 times higher among aquatic plants than over bare mud (Lambret 2016a). Although *L. macrostigma* oviposits in various plant species (see *Reproductive behaviour*), it is closely associated with the Sea club-rush *Bolboschoenus maritimus* (Matushkina et al. 2016). Yet, adults use nearby *Phragmites australis* reed beds and *Tamarix gallica* bushes during maturation, for hunting or to shelter when the wind is strong enough to impair activity at the reproductive site. A common feature of the waterbodies where *L. macrostigma* reproduces is their summer dry period, and the presence of water during spring (larval development period), which largely relies upon rainfall. In addition, the species survey launched across

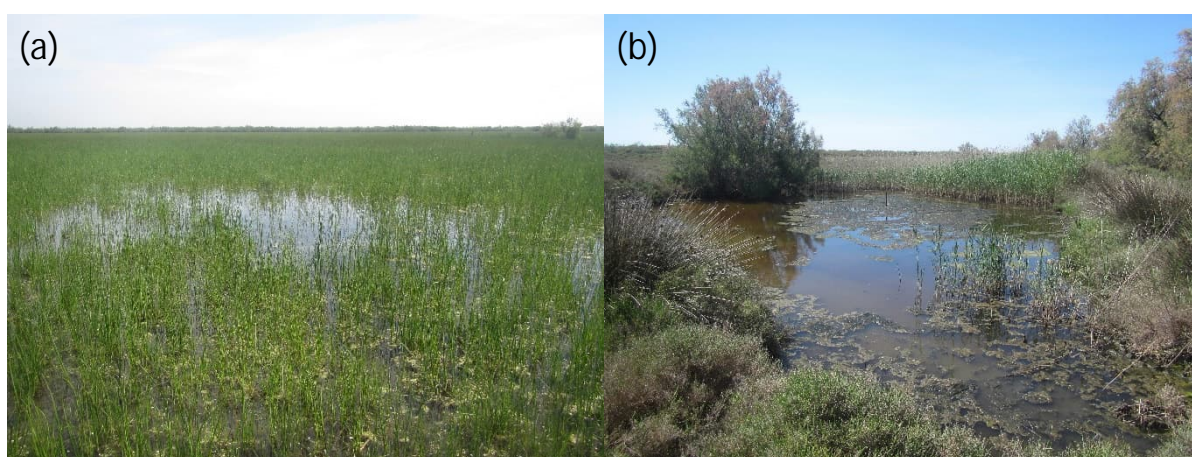


Figure I.8 – Examples of temporary brackish waterbodies where *Lestes macrostigma* reproduces: (a) the marsh “Étang Redon” (43°28’22”N | 04°39’18”E, ca. 27 ha, Tour du Valat Regional Natural Reserve); (b) the pond “Trou du Héron” (43°30’51”N | 04°47’01”E, ca. 335 m<sup>2</sup>, Marais du Vigueirat National Natural Reserve) (© P. Lambret).



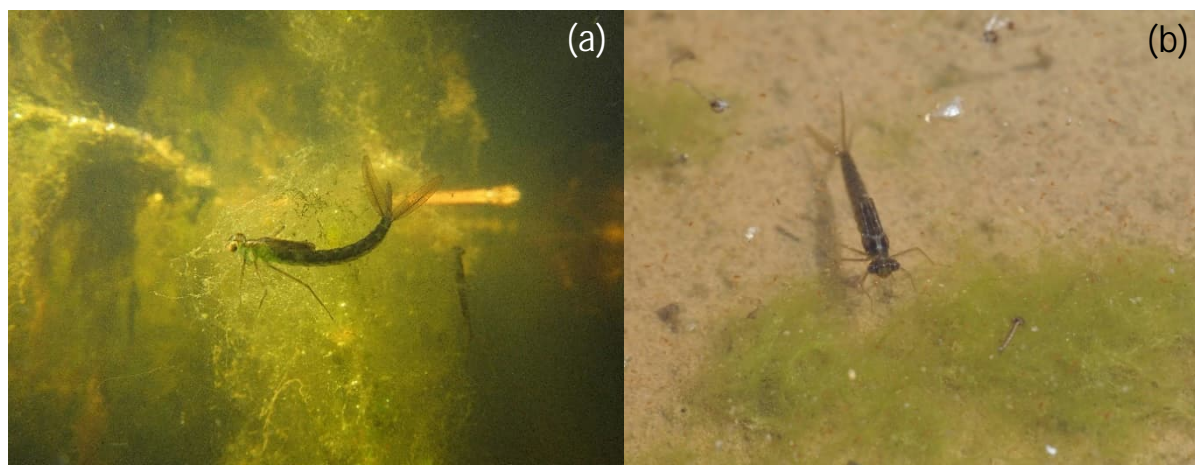


Figure I.9 – Larvae of *Lestes macrostigma* are plant-dwellers (a), and prefer aquatic plants over bare mud (b) (© P. Lambret).

the Camargue in 2010 showed that other larval habitat features (e.g. water depth, salinity, aquatic plants, oviposition plants) were variable, highlighting the need to identify the key environmental factors driving the presence and abundance of larvae, which was addressed in Chapter 4.

#### *Life cycle, diet and population dynamics*

Like every *Lestes* species (Jödicke 1997; Corbet et al. 2006; but see Lambret et al. 2023c), *L. macrostigma* is univoltine (Fig. I.10). Adults are observed during spring and early summer, with the specific flying season depending on location and climate (Dijkstra & Lewington 2006). In the Camargue, emergence typically begins during the first fortnight of May and lasts 2–3 weeks (Lambret 2010; Fig. I.11a). This makes this period ideal for the survey of successful reproduction at known sites and investigation of new breeding sites. The pre-reproductive period (sexual maturation) lasts for ca. two weeks. As odonates are opportunistic predators both at the larval and adult stages (Corbet 2004), adults feed during this period – but also during the rest of their life – on various prey (Fig. I.12a–c). Yet, adults are also prey for numerous predators (Fig. I.12d–g). Adult numbers peak at the beginning of June and adults remain on the wing until late June or early July (Fig. I.11b). The oldest individual recorded in the Camargue using the capture-mark-recapture method had a lifespan of 50 days (Lambret unpubl.). Such a long lifespan is congruent with the literature about other lestid species (see Jödicke 1997). Monitoring the abundance of adults at one site from one year to another is therefore best done during early June in the Camargue. Embryos remain in the plant tissues until larvae hatch the next spring; in other words, the species aestivates and overwinters at the egg stage (Martynov & Martynov 2007; Schiel & Buchwald 2015a). However, detailed information regarding egg and larval development and mortality were still lacking, and were addressed in Chapters 2, 3 & 5.



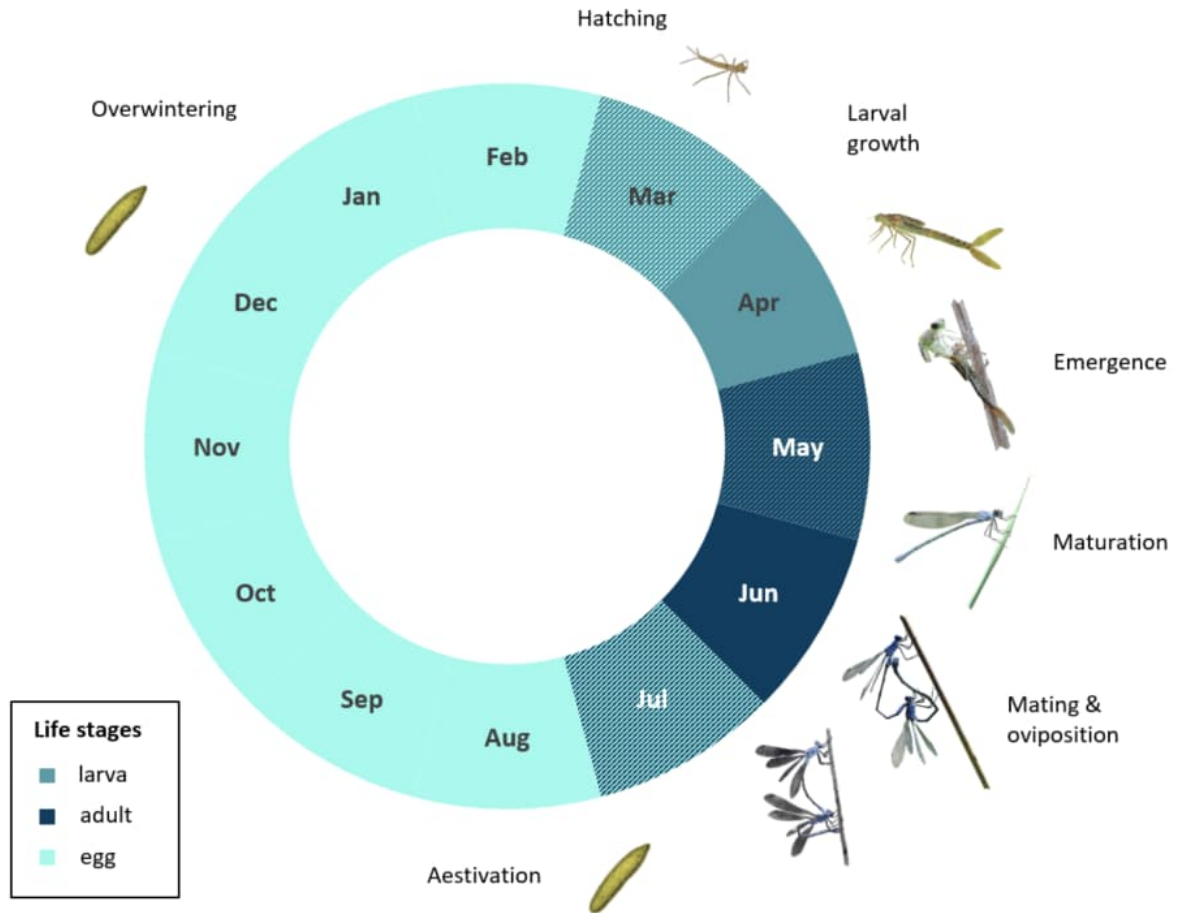


Figure I.10 – Life cycle of *Lestes macrostigma*.

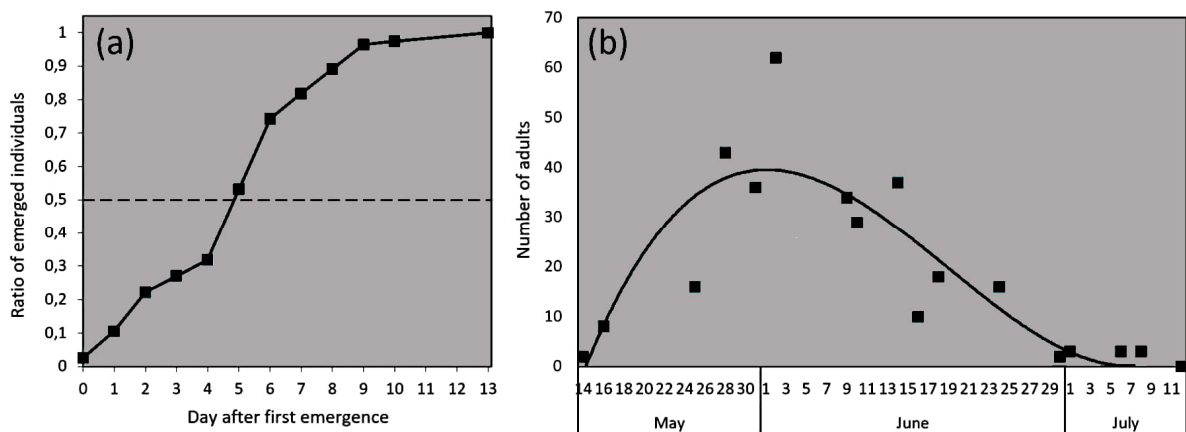


Figure I.11 – *Lestes macrostigma* adult population dynamics at one temporary pond of the Marais du Vigueirat nature reserve (Camargue, France) in 2009 (after Lambret 2010). (a) Emergence curve with the dotted line indicating when 50% of adults have emerged (ca. five days after the 1<sup>st</sup> emergence). (b) Temporal pattern of adult numbers during the flight period.



Figure I.12 – Adults of *Lestes macrostigma* can feed on small prey such as Coleoptera (a) or larger prey such as Tipulidae (b) or other damselflies such as *Ischnura elegans* (c). Yet, they are themselves prey at the teneral stage (e.g. eaten by *Gerris* sp. after falling on the water; d) and at the sexually mature stage, especially with web spiders Araneidae (e); they may also be captured by standing hunting spiders (e.g. Thomisidae) when landing (f) or in flight by other odonates (e.g. *Orthetrum albistylum*; g) (© P. Lambret).

The species experiences strong fluctuations in population size across years where sites may harbour large numbers in one year (Fig. I.13) but very low numbers, or even none, in the next (e.g. Borisov 2005; Lambret et al. 2009; Cano-Villegas & Conesa-García 2009; Berquier & Andrei-Ruiz 2019). Spring rain conditions drive, at least partly, these fluctuations (Frutos Cuadrado et al. 2019; Berquier et al. 2022). During years with high population numbers, when many adults emerge, the species clearly exhibits dispersive behaviour (Papazian 1995). Vagrants are regularly found far, possibly hundreds kilometers, from the nearest reproductive site (e.g. Kuhn 1998; Marinov 2007; Frutos Cuadrado et al. 2019). The species can emerge from numerous sites in a region where reproduction has been successful at 2–3 sites only the previous year (Lambret pers. obs.), showing that dispersal also occurs during low-density years. *Lestes macrostigma* inhabits temporary waters, which have by nature a varying hydroperiod, and therefore constitute a risky habitat for aquatic larvae. In addition, rainfall is highly unpredictable and localized in the Mediterranean area. Hence, a suitable breeding habitat in one year may not be suitable next years. This may explain the evolution of strong adult dispersal behaviour as a strategy to maintain a regional metapopulation.

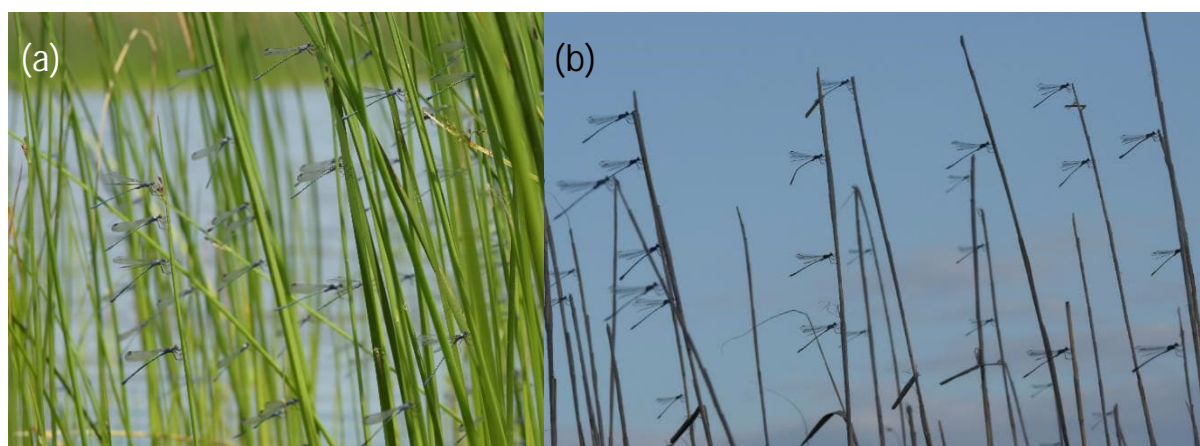


Figure I.13 – *Lestes macrostigma* emerging in high numbers, leading to high density of teneral and maturing adults in the surrounding vegetation (a); in the evening, in order to hunt, adults stand in the upper part of the reed bed where they have sheltered for sexual maturation (b) (Pomorie Lagoon, Bulgaria, a site that harboured many thousands of adults in 2021, 2022 and 2023; © P. Lambret).

#### *Adults' daily pattern of activity*

Monitoring methods recommend gathering data during the maximal activity period of adults. Yet, dragonfly 'activity' is ambiguous and its intensity can be assessed in different ways (e.g. specific behaviour frequencies, flight duration, abundance), including 'responsiveness' (i.e. a threshold of response to a disturbance; see Corbet & May 2008) which was assumed to testify of individuals

'awareness' (Lambret & Stoquert 2011). The daily pattern of activity of *L. macrostigma* mature adults, which features especially reproduction but also feeding and roosting, has an effect on the species detectability (Lambret & Stoquert 2011). Weather may also affect habitat use, as during windy days, adults tend to shelter into reed beds or among *Tamarix* trees, showing the importance of the surrounding landscape. Flight activity of mature *L. macrostigma* is more intense (1) in the early morning in males when they are actively searching for a mate, (2) around midday for pairs (during oviposition), and (3) in the evening for males and females when they feed (Fig. I.12a–c). This makes the temporal pattern of apparent abundance at a breeding site trimodal, yet with highest apparent number of individuals during the early morning. Flight speeds are highest around midday when ambient temperatures are higher. Yet, awareness is almost constant all day long (Lambret & Stoquert 2011). Lambret (2013) showed that also emergence, despite occurring all day long, is more frequent in the morning.

### *Reproduction behaviour*

Although most of pairs are already formed in early morning (Fig. I.14a), they only start copulating in late morning (Lambret & Stoquert 2011). After sperm-transfert (Fig. I.14b), copulation starts and lasts from 10 to 50 min (Chelmick and Lambret 2020), during which the wheel (cover picture) can split and close several times (Fig. I.14c). After copulation, the male most often continue to seize its mate and oviposition starts (Lambret & Stoquert 2011). Females of *L. macrostigma* are endophytic egg-laying odonates and therefore use a cutting ovipositor to insert their eggs inside the plant tissues (Matushkina & Lambret 2011; Fig. I.14e). Oviposition is mostly performed in vertical shoots, but also in broken and therefore horizontal or oblique directed shoots; underwater oviposition has never been observed. Eggs spend the drought season inside the plant shoot they have been laid in (Fig. I.14f) and the desiccation risk may vary depending on the plant species. Indeed, *L. macrostigma* has been most often reported to lay eggs into the Sea club-rush *Bolboschoenus maritimus* (Cyperaceae) (e.g. Stark 1980; Lambret et al. 2009; Schweighofer et al. 2010). Yet, eggs are also laid within other plants, including living shoots of *Carex stenophylla*, *Schoenoplectus lacustris*, *S. tabernaemontani* and *S. litoralis* (Cyperaceae), living and dead shoots of *Juncus maritimus* and dead shoots of *J. acutus* (Juncaceae), dead shoots of *Phragmites australis* (Poaceae) and dead branches of *Tamarix gallica* (Tamaricaceae) (Stark 1980; Martynov & Martynov 2007; Matushkina et al. 2016; Lambret et al. 2023a).

In endophytic egg-laying species, oviposition site selection consists of three crucial steps: (1) initial choice, (2) ovipositor insertion site choice, and (3) egg deposition per se. (1) The initial choice is first decided by the male when landing on the plant material (Fig. I.14d), and then by the female by staying on the substrate or initiating the pair to fly to another. Male *L. macrostigma* prefer to land on *B. maritimus* and dead *Juncus* spp (Lambret et al. 2015a). Focusing on *J. maritimus*, females preferentially palpate the substrate when the male landed on dead shoots. (2) The insertion site choice is made by the female. She bends the abdomen in so as to lean her ovipositor covered by different sensilla against the plant surface (Fig. I.14e, I.15a) in order to palpate the substrate, thereby getting information to choose the insertion site (Matushkina & Lambret 2011; Lambret et al. 2015a). The male preferences are consistent with those of the female during insertion site choice but also during egg deposition (Lambret et al. 2015b). This reduces the duration of the oviposition bout, with reducing predation risk and increasing foraging time. I hypothesized that these advantages for the adults in preferring some substrate should be linked to a selection pressure acting on egg performance, which was tested in Chapter 4. (3) During ovipositor insertion, the female slightly swings and rotates its abdomen from side to side (Fig. I.15b). During this step, the female gets further information about plant stiffness and anatomy, and may abort her attempt to lay one egg (Matushkina et al. 2016). When the cutting valves become entirely inserted in the plant, the female stays motionless for a couple of seconds, only moving the tip of its abdomen at very low amplitude. The egg is laid into the prepared hole probably during this time. Then, the female removes the cutting valves from the hole and walk a few steps backward. These movement patterns are repeated and lead to the insertion of egg clutches in rows along the long axis of the plant (Fig. I.15b, c). The number of eggs of a single clutch is very variable from one clutch to another and is on average  $4.4 \text{ eggs} \pm 5.7 \text{ (SD, min = 1, max = 53, n = 155 clutches; Lambret et al. 2015b; Lambret unpubl.)}$ .



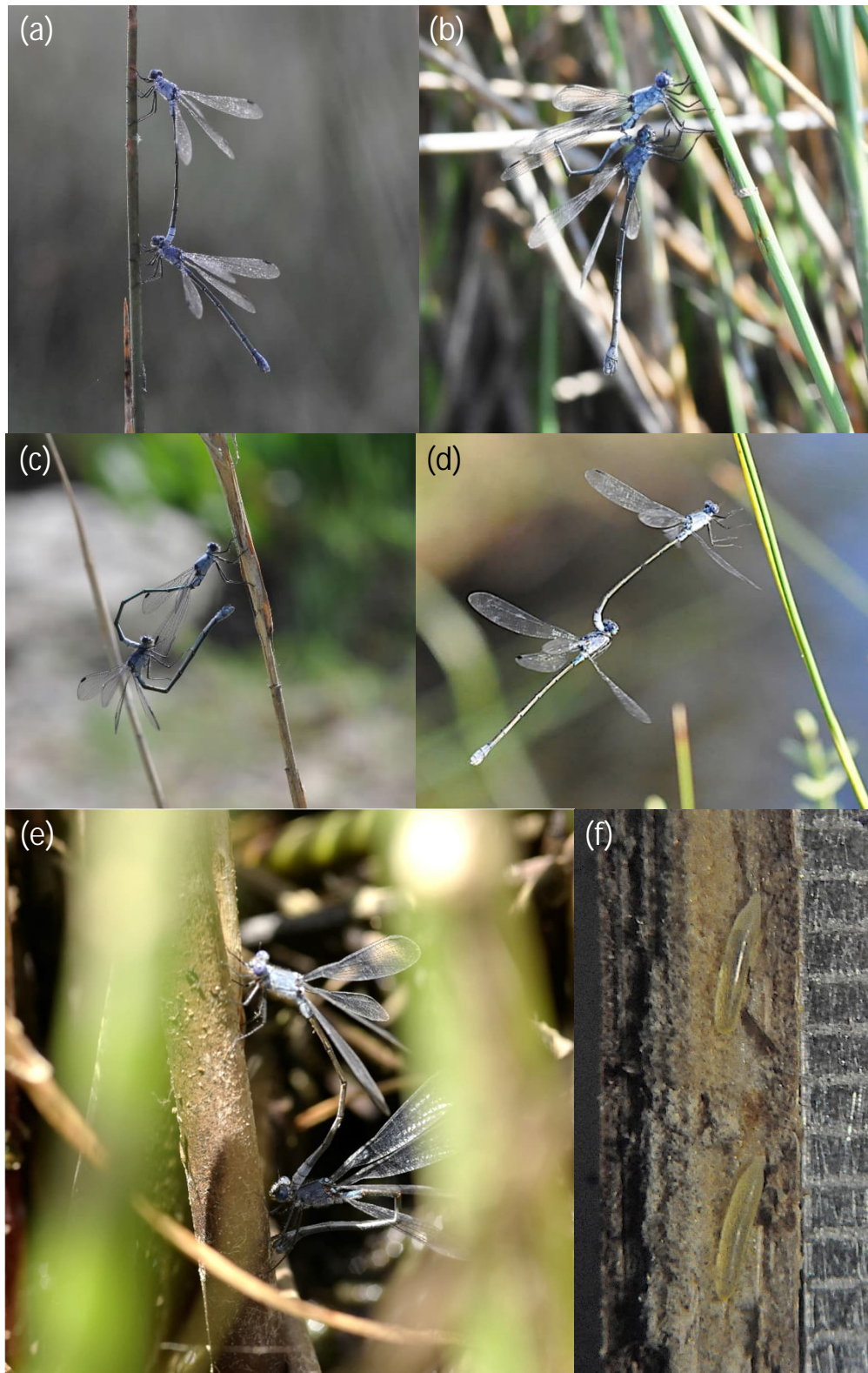


Figure I.14 – Reproduction behaviour of *Lestes macrostigma*: (a) pairs are formed from early morning; (b) sperm transfer from the gonopore to the secondary genitalia (incl. penis); (c) closing of the mating wheel; (d) initial choice of the oviposition site by the male landing on a plant shoot; (e) egg deposition in a dead shoot of *Schoenoplectus tabernaemontani*; (f) dissected dead shoot showing freshly laid eggs (left scale: half millimetres (© P. Lambret).

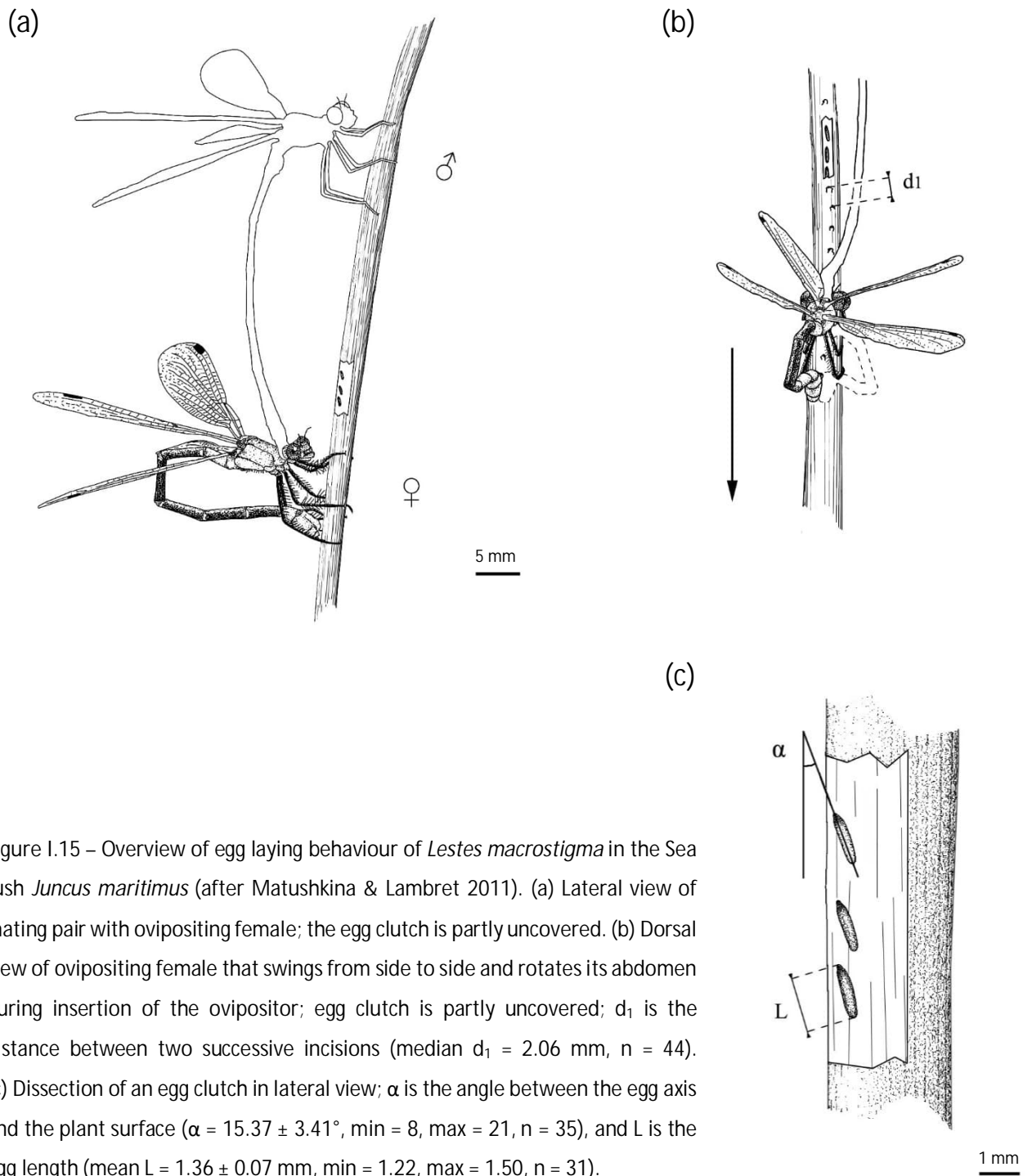


Figure I.15 – Overview of egg laying behaviour of *Lestes macrostigma* in the Sea rush *Juncus maritimus* (after Matushkina & Lambret 2011). (a) Lateral view of mating pair with ovipositing female; the egg clutch is partly uncovered. (b) Dorsal view of ovipositing female that swings from side to side and rotates its abdomen during insertion of the ovipositor; egg clutch is partly uncovered;  $d_1$  is the distance between two successive incisions (median  $d_1 = 2.06$  mm,  $n = 44$ ). (c) Dissection of an egg clutch in lateral view;  $\alpha$  is the angle between the egg axis and the plant surface ( $\alpha = 15.37 \pm 3.41^\circ$ , min = 8, max = 21,  $n = 35$ ), and  $L$  is the egg length (mean  $L = 1.36 \pm 0.07$  mm, min = 1.22, max = 1.50,  $n = 31$ ).

### *Outline of the thesis*

The goal of this work is to improve the conservation status of *Lestes macrostigma* and to use this species as a flagship to enhance the launch of restoration programs for MTPs. Hence, my thesis aimed at filling in several important conservation-related knowledge gaps in the biology of this species. In the previous part (Study species), mostly relying on my own research in the Camargue, I provided details on the biology of the study species, focusing on those having implications for its identification, monitoring and conservation. This part was not a review per se and only the relevant information for the conservation of the species and of temporary brackish ponds were provided. This part highlights several important gaps of knowledge, especially regarding two life-history stages for odonates' population dynamics: the egg and the larval stages (McPeck 2008). I aimed at answering the four following questions: (1) Which factors drive egg survival and embryonic development? (2) Which factors drive larval density and fitness? (3) Which factors drive adult fitness and size at emergence? (4) Does the oviposition preference by adults for the Sea club-rush *Bolboschoenus maritimus* benefit their progeny? In Chapter 1, I studied the development of the embryo, from egg laying to hatching. I assessed hatching success in two temporary ponds to investigate the effect of cold temperature, i.e. embedment in ice, which is directly related to winter water levels. In Chapter 2, I investigated the effect of salinity on egg hatching success by exposing embryos to three salinity levels (2, 5.5 and 9.5 g/L), and on lethal and sublethal life-history and physiological effects during larval growth and at metamorphosis by exposing larvae to four salinity levels (0.5, 4, 8 and 16 g/L). Chapter 3 identified the influence of biotic and abiotic variables on larval densities based on a field study conducted in 33 temporary ponds across the Camargue. In Chapter 4, I studied the consequences of adults' plant preference during oviposition for their progeny fitness. For this, I carried out under semi-natural conditions two experiments with plant shoots containing eggs of *L. macrostigma* by manipulating flooding dates and shoot types and looked for effects on hatching success and hatching dates. Hence, the answers to the questions asked above can be found across these different chapters (Fig. I.16). In the General discussion, I replaced this work in the frame of the conservation of threatened insects and related research. Then I integrated the results of all chapters and translated these into recommendations for the conservation management of *L. macrostigma* and for designing restoration programs targeting this species and temporary brackish ponds. This part is illustrated by pilot programs I implemented in the Camargue.



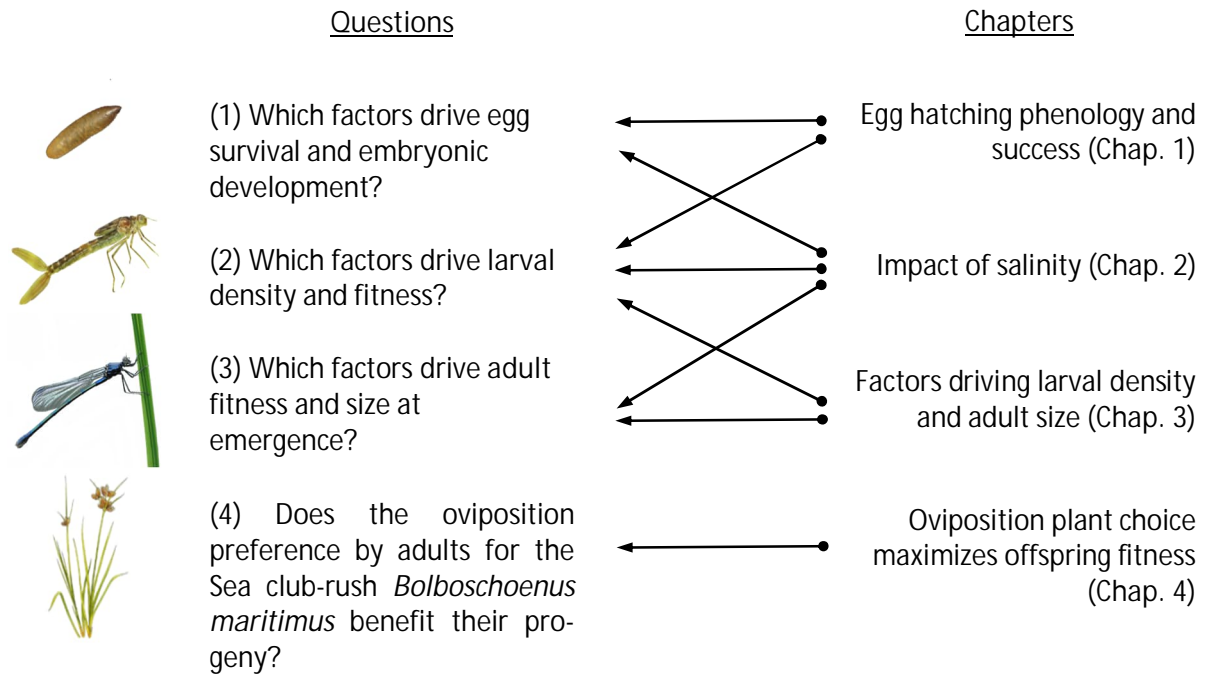


Figure I.16 – Diagram showing how the four chapters of this PhD fill the knowledge gaps (questions) about *Lestes macrostigma* ecology.

## Chapter 1

### Egg hatching phenology and success of *Lestes macrostigma* in two temporary brackish ponds

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Slightly adapted

#### *Abstract*

Although a full life cycle approach is optimally needed to make conservation decisions, the egg stage is often neglected for insect species of special conservation interest. Water management and related abiotic factors are relevant to consider in aquatic species. *Lestes macrostigma* is a threatened damselfly restricted to temporary brackish waters. Here we provide detailed information on its hatching success and phenology in two natural field populations. Shoots containing fresh egg clutches of *L. macrostigma* were sampled in late June, just after the oviposition period. In the autumn, shoots were separately placed in plastic boxes in two ponds in southern France. Examination of eggs indicated *L. macrostigma* overwinters at an early embryonic stage. The following spring we monitored hatching in detail. Hatching began on 15 March in both ponds and ended on 27 April. Hatching was synchronized with half of the eggs hatching within five days in the first pond and 14 days in the second pond. Lower water temperatures decreased hatching success and likely also delayed hatching. Embedment of shoots in ice increased egg mortality. Based on our data, wildlife managers are encouraged to maintain water levels high during winter to reduce the risk of freezing of *L. macrostigma* eggs.

## Introduction

In addition to habitat protection, the knowledge of life-history is of primary importance to design conservation programs for endangered insect species (e.g. New 2012; Stewart et al. 2007). A full life cycle approach is needed to optimally make conservation decisions (Thompson et al. 2003). However, most studies on species of special conservation value focus only on larval and/or adult stages (e.g. Leipelt & Suhling 2001; Ohba & Inatani 2012; Wildermuth 2008). In contrast, studies concerning the egg stage in damselflies have been limited to common species (e.g. Koch 2015; Śniegula et al. 2016). Yet, studies on insect demography have clearly indicated that egg survival, which may depend on environmental factors such as oxygenation and desiccation, can contribute considerably to population dynamics (Pasquier-Barre et al. 2001; Siva-Jothy et al. 1995). Water levels and related abiotic factors, especially water temperatures (including freezing), are likely to affect the egg survival and phenology of endangered species (Lahr et al. 1999; Sawchyn & Gillott 1974a). Management of the water levels in ponds is therefore relevant to the conservation of aquatic insects (Samways et al. 2010).

Odonates have received increased attention in conservation programs (e.g. Harabiš & Dolný 2015; Raebel et al. 2012; Reece & McIntyre 2009). Due to their aquatic habitat and above all their smaller size, eggs and first instar larvae have mainly been studied in laboratory or semi-natural conditions (e.g. Lestidae: Sawchyn & Church 1973; Coenagrionidae: Van Doorslaer & Stoks 2005; Libellulidae: Miller 1992). By contrast, studies focusing on egg hatching in natural conditions remain rare in odonates, and are mainly limited to non-quantitative descriptions (Pierre 1904; Sawchyn & Gillot 1974a; Tab. 1.1), precluding the assessment of hatching patterns (but see De Block et al. 2005) and egg mortality in natural populations.

*Lestes macrostigma* (Eversmann, 1836) is a damselfly species which requires particular conservation attention (Dupont 2010). This lestid species is stenoecic and restricted to temporary brackish (oligohaline) waters, especially in the western part of its distribution range (Boudot & Kalkman 2015). The disappearance of this habitat has led to the former threatened and now 'Near Threatened' conservation status of *L. macrostigma* within the EU (Kalkman et al. 2010; G. de Knijf pers. comm. 2024). The species biology is extensively studied, including the larval development (Schiel & Buchwald 2015a) and the adult population dynamics (e.g. Borisov 2005; Lambret 2010). Yet, much less is known about the egg stage.

Egg clutches are laid by *L. macrostigma* females during spring in plant parts above the water surface and are typically flooded by rainfall in autumn and winter (Lambret et al. 2015b; Matushkina & Lambret 2011). Similar to other *Lestes* species (Jödicke 1997), the species overwinters in the egg

Table 1.1 – Embryonic characteristics of *Lestes* damselfly species with an overwintering egg stage: the diapausing egg type and hatching phenology. Egg type was 1 or 2 according to Corbet (2004). Study type was coded as lab (laboratory conditions), s-nat (semi-natural conditions) and fld (field observations). Hatching period, duration, range R (number of days between 25% and 75% quartile of hatchings) and H<sub>50</sub> (number of days elapsed since hatching began) are given if available from field or semi-natural conditions.

Species	Latitude N	Egg type	Type of study	Beginning of hatching period	Duration of hatching period (days)	50% hatching (total number of hatchlings)	References
<i>L. barbarus</i> (Fabricius, 1798)	48°38'	type 2	s-nat	mid-Jan. to early April	3–6	R = 4–9 (254–406)	Schiel & Buchwald 2015a; F.J. Schiel pers. comm.
<i>L. d. disjunctus</i> Selys, 1862	49°20' to 50°26'	type 2	lab & fld	early to mid-May	7		Sawchyn & Church 1973; Sawchyn & Gillot 1974a; Duffy 1994
<i>L. d. australis</i> Walker, 1862	35°05'		fld	mid-June			Ingram 1976
<i>L. dryas</i> Kirby, 1890	48°38'	type 2	lab & s-nat	early Jan.	59	R = 5 (242)	Sawchyn & Church 1973; Schiel & Buchwald 2015a
<i>L. sponsa</i> (Hansemann, 1823)	48°38' to 51°23'	type 2	s-nat & fld	late Jan. to mid-April	93	R = 26 (154)	Schiel & Buchwald 2015a; Corbet 1956a, 1956b
<i>L. unguiculatus</i> Hagen, 1861	50°26'	type 2	lab & fld	early May	7		Sawchyn & Church 1973; Sawchyn & Gillot 1974a
<i>L. rectangularis</i> Say, 1839	41°39'	type 1 & 2	lab & fld	early April	12		Gower & Kormondy 1963
<i>L. congener</i> Hagen, 1861	52°15'	type 1	lab & fld	late May	7		Sawchyn & Gillot 1974b
<i>L. macrostigma</i> (Eversmann, 1836)	43°30'	type 1	fld	mid-March	37–43	H <sub>50</sub> = 4–13 (578–701)	Lambret et al. (this study)
	47°07' to 48°51'		lab	mid-March	30		Martynov & Martynov 2008
	48°38'		s-nat	mid-March	16	R = 3 (12)	Schiel & Buchwald 2015a
<i>L. virens</i> (Charpentier, 1825)	48°38'	type 1	s-nat	late March to early April	46–58	R = 6–15 (699–1,443)	Schiel & Buchwald 2015b; U. Norling pers. comm.
<i>Chalcolestes viridis</i> (Vander Linden, 1825)	51°17'	type 1?	fld	early April	35–45	R = 6–14 (908–23,430)	Münchberg 1933; De Block et al. 2005

stage and shows an obligate diapause before hatching (Schiel & Buchwald 2015b). Wintering damselfly eggs are divided into two types: eggs belonging to type 1 winter at an early development stage, before katatrepsis, and those belonging to type 2 winter in an almost fully completed embryonic stage, after katatrepsis (Corbet 2004). *Lestes* species belong to both types 1 or 2 (Corbet 2004) but this life-history trait is unknown in *L. macrostigma*. Eggs hatch in March–April (Martynov & Martynov 2008; Schiel & Buchwald 2015b). Hatching synchronization and success increase together with the duration of periods of low temperature (10 °C) during winter (Aguesse 1961). However, there is a lack of information regarding egg hatching phenology and egg mortality, and how these are affected by abiotic conditions and freezing.

In this study, we documented the embryonic development stage during diapause, and quantified the egg hatching phenology and egg mortality in two natural *L. macrostigma* populations. In order to explore inter-population variation in these demographic variables, we studied this at two ponds. In addition, we tested whether egg survival was impacted by embedment of the shoots in the ice.

## *Material and methods*

### *Study site and monitoring of abiotic variables*

The study took place in the National Natural Reserve of the Marais du Vigueirat, a protected wetland area in southern France. The experiment was run in two brackish ponds: (1) the Baisse des Marcells (43°29'27"N | 04°48'22"E) is a pond of ca. 1.5 ha within a larger swamp, surrounded by dams and relatively exposed to the Mistral (a cold northern wind); (2) the Trou du Héron (43°30'51"N | 04°47'01"E) is a man-made pool of 335 m<sup>2</sup>, relatively sheltered from the Mistral by *Tamarix gallica* trees. These ponds are hereafter named BdM and TdH, respectively. The TdH pond is only fed by rain, while the BdM pond receives additional fresh water input from a canal from October to April. Both ponds dry out due to progressive evaporation during the rest of the year. Given the high annual water evaporation levels in the Mediterranean, both ponds have extended dry periods. For example in 2011, BdM and TdH were respectively flooded the last week of October and the first week of November, while by the second week of July and the last week of June 2012 both ponds were completely dry.

Water temperatures and conductivity (a proxy for salinity, Waterkeyn et al. 2010) were monitored throughout the period that shoots with eggs were in the water (from December 2011 to May 2012, Tab. 1.2). Water temperature and conductivity were measured each week (on days without wind) between 14:00 and 16:00 (local time) in both ponds using a portable meter WTW® Cond 315i (Weilheim, Germany). Weekly minimal and maximal water temperatures were also recorded using

thermometers that were maintained near the boxes containing the eggs (see below). Readings are missing for several weeks due to problems with the min–max thermometers (Tab. 1.2). Mean daily air temperature and photoperiods (duration from sunrise to sunset) were calculated using data from the meteorological station of ‘Arles-Valat’ (Météo France, station n°13004003, 43°30’36’’N | 04°41’38’’E) at ca. 7 and 9 km from the study ponds.

### *Experimental procedure*

Dry shoots of different plant species that contained egg clutches of *L. macrostigma* were sampled at the end of June 2011: *Bolboschoenus maritimus* and *Juncus maritimus* at BdM, and *J. acutus* at TdH. Shoots containing *Lestes* eggs were identified using the oviposition incisions made by the female ovipositor (Matushkina & Lambret 2011; McMillan & Arnold 2003). At TdH no other *Lestes* species were present; at BdM, *L. sponsa* (Hansemann, 1823) was also present. Shoots containing *L. macrostigma* eggs were sorted from those containing *L. sponsa* eggs using the location and insertion pattern of the egg clutches: *L. macrostigma* females oviposit at the bottom of the shoot and the median distance between two successive oviposition incisions is 2.1 mm ( $n_{\text{distances}} = 44$ ,  $n_{\text{shoots}} = 4$ ) (Matushkina & Lambret 2011). *Lestes sponsa* females oviposit at the top of *J. maritimus* shoot and the median distance between two successive oviposition incisions is 9.6 mm ( $n_{\text{distances}} = 30$ ,  $n_{\text{shoots}} = 7$ ).

To mimic the natural conditions in temporary ponds, dry shoots with eggs were placed vertically into 10 × 20 × 20 cm blocks of styrofoam and kept outdoors at the National Natural Reserve under natural weather conditions during summer and autumn. Blocks of styrofoam were placed in a 40 × 50 × 100 cm metal cage to prevent any damage caused by rodents or wild boars. To assess the embryonic stage at which eggs enter winter diapause we dissected some shoots collected in autumn and examined eggs with a Novex 65.560 RZT-SF stereomicroscope. In December, single intact shoots with eggs were placed into separate 5 × 16 × 24 cm plastic boxes, each of which was labelled (Fig. 1.1b). This allowed monitoring of egg hatching for each shoot separately. In order to allow water to enter the box without the freshly hatched larvae escaping, we made a hole (9 cm diameter) in one side of the box and covered it with a 0.25 mm mesh tissue (Fig. 1.1b). Boxes were placed in the pond by gently immersing each them, hole to the top, to flush the air they contained. Boxes were randomly positioned ca 15–20 cm under the water surface in each pond using a chicken wire cage and a floater (Fig. 1.1a). In BdM, boxes were moved to a deeper part of the pond at the second week of February

Table 1.2 – Overview of the monitored abiotic variables during the period eggs were present in the two study ponds: water depth (cm), conductivity (a proxy for salinity, mS/cm) and temperature (°C). Light grey shading indicates the egg hatching period. \* indicates the week of egg embedment in ice at BdM. Note that several minima and maxima temperatures could not be recorded due to problems with the min–max thermometers.

Year	Month	Week	Water depth		Conductivity		Temperature					
			BdM	TdH	BdM	TdH	Afternoon		Week min		Week max	
							BdM	TdH	BdM	TdH	BdM	TdH
2011	Dec	50	22	58	3.31	3.01	10.5	9.5				
		51	20	56		1.66		4.6				
		52	19	54	3.70	4.90	5.7	5.9				
2012	Jan	01	19	54	4.18	5.21	9.9	8.8	1		12	
		02	19	53	4.33	5.88	6.9	7	4	7	9	7
		03	18	46	4.01	5.57	3.8	4.8				
		04	18	52	4.63	7.02	9	8.6	1.5	6	11	10
	Feb	05	16	51	4.26	7.56	6	6.5	4.5	7	10	10
		06	20	50	5.95	10.02	3	1.7	1.5	3	4.5	7
		07*	10	50		10.84		3.5		3.5		4
		08	76	49	4.72	9.43	6.9	6.8		4		10
		09	80	45	5.40	10.39	10.8	11		8		13
	Mar	10	77	43	5.67	8.38	11.3	11.1		6		18
		11	79	41	6.42	11.31	15.9	14		11		17
		12	81	42	5.62	11.44	16.9	14.6		14.5		18
		13	81	39	5.70	11.80	17.7	19.4	11.5		20	20
	Apr	14	82	38	7.27	12.59	18	18.3	13.5		20	23
		15	82	34	5.60	12.78	18.5	16.2	11		20	22
		16	81	31	5.29	13.59	14.4	17.5	18		20	21
		17	76	26	5.79	14.17	16.9	20.6	8	11	18	23
		18	84	25	3.94	14.55	16.7	16.9	13	15	20	23
	May	19	83	24	4.01	15.31	21.1	23.5	16	18	21	27

(freezing episode), since water depth had decreased to 10 cm (Tab. 1.2). Since shoots did not contain the same number of eggs, boxes with shoots were distributed across both ponds to balance the number of eggs as evenly as possible regarding the plant species they had been laid in (Tab. 1.3).

The presence of hatched larvae was checked in each box every week from February 2012 onwards, and every 2–3 days once the first hatched larva was detected. During each visit, all hatched larvae were counted in each box and removed (Fig. 1.1c). To calculate egg hatching success we counted the number of oviposition incisions per shoot (Tab. 1.3), thereby assuming each of these corresponds with one egg laid (Matushkina & Lambret 2011). The estimates obtained by this method

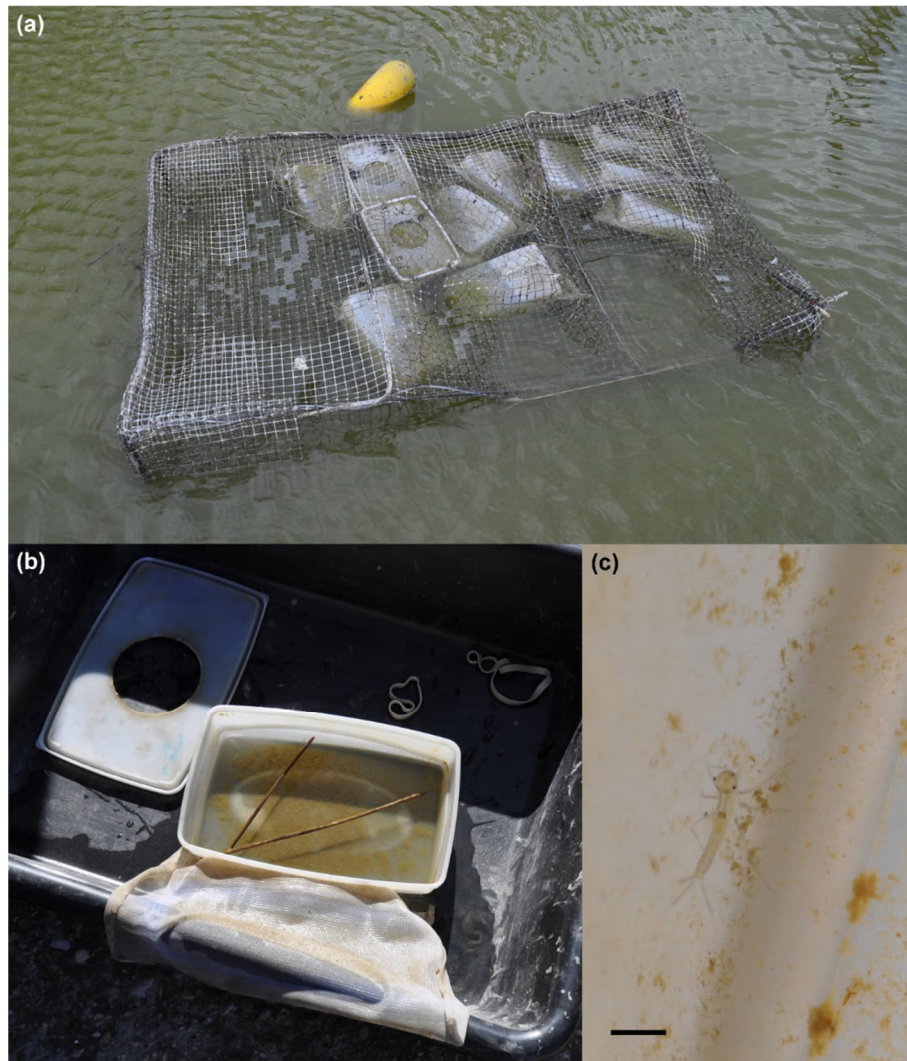


Figure 1.1 – Experimental setup for the monitoring of *Lestes macrostigma* hatching under field conditions: (a) the cage containing boxes; (b) a box containing one shoot with eggs; (c) one hatchling inside the box (scale bar = 1 mm).

may be slightly biased. On the one hand, females sometimes make incisions without laying eggs (Gower & Kormondy 1963; Lambret et al. 2015b), inducing an overestimation of the number of laid eggs based on incision number. In order to minimize this bias, since eggs are deposited only after longer incision and full protrusion of the ovipositor (Martens 1992, 2001; Lambret et al. 2015b), we avoided counting small incisions. On the other hand, there were cases where we counted more hatched larvae than oviposition incisions in some shoots, indicating we occasionally failed to detect oviposition incisions. In such shoots, the corresponding hatching success was set at 100%.



### *Statistical analyses*

Differences in water temperature and conductivity between ponds were tested using the Wilcoxon rank test for paired values. Differences in hatching success and hatching date were tested using Generalized Linear Mixed Models (GLMMs). Significance was determined by analysis of deviance. Ponds were included in models as a fixed factor. Embedment in ice was included as a fixed factor when testing for its effect and as random effect when testing for differences between ponds in hatching phenology and success. Since hatching success and hatching date may depend on plant species (Grunert 1995), plant species was included as a random effect. In addition, box (i.e. shoot) was also included as random effect in the models.

Hatching success (proportion of eggs hatched per shoot) was analyzed using a binomial error structure (Zuur et al. 2009). Hatching dates were expressed as log (Julian dates), with day one being 1 January 2012, and analyzed using a Gaussian error structure. We checked model assumptions (the normality, the heteroscedasticity and independence of the residuals) following Zuur et al. (2009). Unless otherwise stated, results are given as a mean with 95% confidence intervals. All analyses were performed with R 2.14 (R Core Team 2020), the GLMM were run with packages 'lme4' (Bates et al. 2019) and 'AICcmodavg' (Mazerolle 2015).

### *Results*

#### *Embryonic developmental stage when entering winter diapause*

All eggs ( $n = 69$ ) collected in autumn were in an early, pre-katatrepis stage. Cleavage had occurred, as indicated by the coarse grained structure of the yolk. Yet, no embryo was visible. Therefore, eggs belong to type 1 according to Corbet (2004) indicating *L. macrostigma* overwinters at an early embryonic developmental stage.

#### *Water temperature and conductivity*

Instant water temperatures closely followed the variation in air temperatures (Fig. 1.2), varying between 1.7 and 23.5 °C, and did not differ between ponds (Wilcoxon matched paired test,  $W = 99.5$ ,  $p = 0.837$ ,  $n_{\text{pairs}} = 20$ ). By contrast, for the subset of weeks where minimum and maximum temperatures were available, the temperatures were lower in BdM than in TdH in 14 out of the 18 weeks (Tab. 1.2). This suggests that the mean water temperature and sum of degree-days were lower in BdM than in TdH, possibly due to the higher exposure to the cold Mistral wind. Conductivity

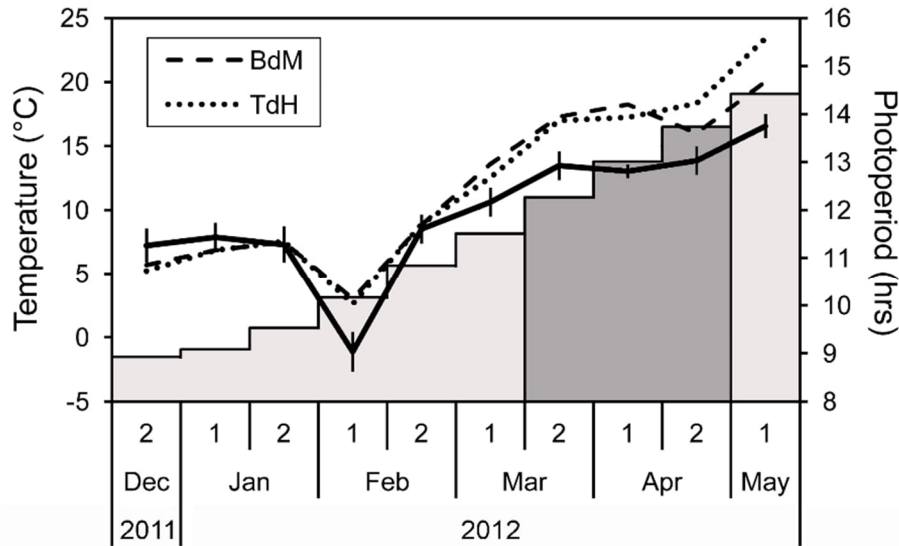


Figure 1.2 – Seasonal (per fortnight) changes in air and water temperature (left axis) and photoperiod without twilight (right axis) when *Lestes macrostigma* eggs were in the water of the study ponds (BdM and TdH). Temperature curves are coded as follows: full line = mean air temperatures (with 95% confidence intervals); dotted lines = windless afternoon instant water temperatures at both ponds. The dark grey shading indicates the egg hatching period at both study sites.

ranged from 1.66 to 15.31 mS/cm (Tab. 1.2) and was significantly higher in TdH (mean = 9.43, SD = 3.88) than in BdM (mean = 4.99, SD = 1.03) (Wilcoxon matched paired test,  $W = 1$ ,  $p < 0.001$ ,  $n_{\text{pairs}} = 20$ ). The observed values indicate mild and brackish saline conditions in BdM and TdH, respectively.

#### *Effect of ice embedment*

There was a cold wave early February 2012 (week 7, Tab. 1.2; Fig. 1.2). At that moment the water level was 10 cm in BdM, and as a result some shoots were close enough to the water surface to be fully embedded in ice for two days. In contrast, at the same time, the water level was 50 cm in TdH and as a result all the shoots in TdH were submerged in water at 15–20 cm below the ice surface. When assessing the effect of ice embedment on egg mortality we therefore restricted our dataset to BdM. Hatching success was reduced by more than 50% when shoots had been embedded in the ice (mean with 95% CI: 26.16% [7.45–60.86],  $n = 188$  eggs) than when this was not the case (55.08% [31.03–76.96],  $n = 580$  eggs). Yet, this effect did not reach statistical significance ( $\chi^2_1 = 1.759$ ,  $p = 0.185$ ). This was due to the high variability between shoots as suggested by the fact that the effect of ice embedment became significant when the ‘box’ random effect was removed from the model ( $\chi^2_1 = 42.062$ ,  $p < 0.001$ ).

Table 1.3 – Median hatching dates and hatching success for each studied plant shoot. Light grey shading indicates shoots that had been embedded in ice.

Pond	Plant sp	Embedment in ice	Median hatching date	Number of oviposition incisions	Number of larvae	Hatching success
TdH	<i>B. maritimus</i>	no	19 March	46	38	82.6%
		no	17 March	31	32	100%
	<i>J. acutus</i>	no	19 March	104	108	100%
		no	17 March	192	102	53.1%
	<i>J. maritimus</i>	no	28 March	361	242	67.0%
		no	17 March	81	38	46.9%
		no	17 March	137	66	48.2%
		no	19 March	123	75	61.0%
BdM	<i>B. maritimus</i>	no	28 March	16	7	43.8%
		no	30 March	26	19	73.1%
	<i>J. acutus</i>	yes	26 March	26	1	3.8%
		yes	28 March	162	95	58.6%
		no	30 March	391	251	64.2%
		no	4 April	147	55	37.4%
	<i>J. maritimus</i>	yes	22 March	36	26	72.2%
		yes	30 March	184	80	43.5%
		yes	28 March	124	44	35.5%

Ice embedment did not affect the egg hatching date ( $\chi^2_1 = 2.350$ ,  $p = 0.125$ ). Half of the eggs that had been embedded in the ice hatched by 28 March (range = 17 March – 11 April,  $n = 96$  eggs) and half of those that had not been embedded hatched by 2 April (range = 17 March – 27 April,  $n = 332$  eggs).

#### Pond differences

Hatching success differed significantly between ponds ( $\chi^2_1 = 4.167$ ,  $p = 0.041$ ): it was higher in TdH (79.18% [58.30–91.19],  $n = 1075$  eggs) than in BdM (46.16% [25.34–68.41],  $n = 1112$  eggs). When excluding shoots embedded in ice, hatching success was still higher in TdH (78.55% [59.15–90.25],  $n = 1075$  eggs) than in BdM (53.49% [30.48–75.11],  $n = 924$  eggs) but no longer significantly so ( $\chi^2_1 = 2.861$ ,  $p = 0.091$ ), supporting the negative effect of embedment in ice on hatching success.

The first eggs hatched on 15 March in both ponds. Despite the higher numbers of eggs and hatched larvae in TdH, hatching was more synchronous than in BdM: 701 larvae hatched in 38 days in TdH vs 578 larvae in 44 days in BdM. Eggs also hatched significantly earlier in TdH than in BdM

( $\chi^2_1 = 17.691$ ,  $p < 0.001$ ; Fig. 1.3). Half of the eggs had hatched by 19 March (range = 15 March – 21 April,  $n = 701$ ) in TdH and nine days later, by 28 March (range = 15 March – 27 April,  $n = 578$ ) in BdM.

## Discussion

### *Egg hatching phenology*

The egg hatching period (March–April) for *L. macrostigma* that we observed in natural conditions in southern France is consistent with that documented in Central Europe (Martynov & Martynov 2008; Schiel & Buchwald 2015b; Tab. 1.1). Despite the long adult reproductive period (ca. 45 days, Lambret 2010) and the extended period during which the eggs had been laid, egg hatching was highly synchronised with up to 50% of the eggs hatching during a two week period. This synchronisation is due to the fact that embryonic development is arrested when embryos reach the same pre-katatrepsis stage in summer (egg diapause). After winter, when temperatures surpass a threshold, egg development is reinitiated in all eggs at the same time (Schiel & Buchwald 2015b). Among dragonflies with diapausing eggs, a high level of synchronisation in egg hatching is typical of species occupying temporary ponds (Schiel & Buchwald 2015a). Temporary pond species are also known to hatch earlier (De Block et al. 2005; Schiel & Buchwald 2015a, 2015b). Such early synchronized hatching (Tab. 1.1) can be explained by selection for hatching as soon as the conditions are favourable for larval development. This allows the species to avoid mortality due to the ponds drying out before the aquatic larval stage can be completed. This is especially relevant as lestid larvae are not able to respond to pond drying by accelerating their development (De Block & Stoks 2005b), thereby matching the general pattern found in aquatic insects inhabiting temporary ponds (Stoks et al. 2014).

Within the European temporary pond *Lestes* species, usually hatching early, *L. macrostigma* hatches the latest, yet has the shortest development time and the highest growth rate (De Block et al. 2005; Schiel & Buchwald 2015a, 2015b, 2016a). This late hatching correlates with the species early diapause stage (egg type 1) and contrasts with other European temporary pond *Lestes* that show egg type 2 (Tab. 1.1). The holomediterranean *L. macrostigma* is singularly restricted to temporary waters (Boudot & Kalkman 2015; Dommanget 1987) with high seasonal variability in flooding dates and duration, especially in the southern part of its distribution range. We hypothesize that the species' strategy to maintain an early diapause stage, and therefore a late hatching date, is adaptive in a typically unpredictable environment thereby avoiding too early hatching when ponds may still be dry in some years. This late hatching is combined with fast larval development and growth to enhance the probability of *L. macrostigma* larvae to reach the adult stage before pond drying.

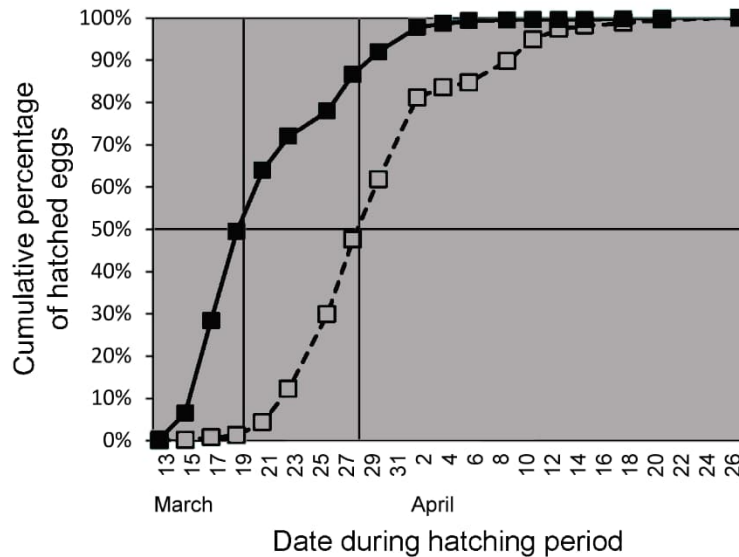


Figure 1.3 – Cumulative percentage of eggs that hatched throughout the hatching period in both study ponds (filled squares: TdH, empty squares: BdM). The crossover points of grey lines indicate the dates when 50% of eggs hatched.

#### *Effects of ice embedment on egg survival*

Our results show that, despite being a typical Mediterranean species, *L. macrostigma* can survive episodes of ice embedment in the egg stage. In the study region, in a period of 14 years (2000 to 2013), subzero temperatures did not occur every year, but only during six years and on three to 12 days. This is consistent with the literature on northern *Lestes* species (Jödicke 1997) and can be explained by the fact that *L. macrostigma* has permanent populations in much colder regions such as Siberia and Austria (Boudot & Kalkman 2015). Yet, our data also suggest that egg hatching success decreased by ca. 50% when eggs had been embedded in the ice. The latter pattern was, however, not significant, probably due to the large variation between the plant shoot replicates and the short duration of ice embedment. In support of a negative embedment effect on hatching success, the pattern became significant when no longer including box (hence shoot) in the model and the lower hatching success in BdM than in TdH was no longer significant when excluding the embedded eggs of BdM (the only pond where eggs were embedded). Although *Lestes* eggs can be extremely resistant to low temperatures, this resistance decreases as the embryonic development advances (Fisher 1964; Sawchyn & Gillot 1974b). Hence, embedment in ice can be regarded as a factor which has a negative impact on the survival of *L. macrostigma* eggs, especially during post-diapause development.

### *Differences between ponds*

Eggs hatched earlier and with a higher synchrony in TdH than in BdM. The differences in salinity between ponds were probably not causing this pattern because the egg membrane protects the developing larva from harmful substances (e.g. Hardersen & Wratten 2000). More likely, differences in water temperature contributed to the differences in egg hatching patterns between both ponds. Post-diapause egg development in damselflies is faster at higher temperatures (e.g. Sawchyn & Gillot 1974b). While water temperatures were similar in both ponds on windless days, we hypothesize that the temperatures were lower in BdM than in TdH on windy days, as suggested by the lower weekly minimum and maximum water temperatures observed in BdM.

### *Implications for conservation*

Our data indicate that water temperatures and especially ice embedment are important factors in shaping the population dynamics of *L. macrostigma*. Wildlife managers can increase winter egg survival by controlling the hydroperiod of temporary ponds. This could be accomplished by ensuring higher water levels during winter to shelter *L. macrostigma* eggs from low temperatures and to avoid eggs being embedded in the ice. High water levels throughout spring may however reduce water temperatures thereby slowing down egg development rates and postponing egg hatching. This may not be a problem, as higher water levels throughout spring would delay pond drying, allowing a longer period for larval development before adult emergence. Our results support the view that considering the egg stage in a full life cycle approach may be rewarding when taking conservation measures (Thompson et al. 2003).

### *Acknowledgements*

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## Chapter 2

### The impact of salinity on a saline water insect: Contrasting survival and energy budget

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Slightly adapted

#### *Abstract*

Water salinity is a major driver of aquatic insects' distribution. Saline species are usually generalists with high survival and performance at both low and high salinity levels. Yet, costs of high salinity may be underestimated as these are most often measured in terms of larval life-history traits, while effects of larval stressors may only be detectable when looking at physiological traits and traits in the adult stage. Here, we assessed the lethal and sublethal physiological effects of embryonic and larval exposure to a range of salinity levels in the damselfly *Lestes macrostigma*, both during and after metamorphosis. This species inhabits temporary freshwaters where salinity increases during the drying phase. Salinity had no effect on egg hatching success within the range 2–9.5 g/L sea salt (conductivity range 3.45–14.52 mS/cm). With increasing salinity (up to 16 g/L, 23.35 mS/cm), growth rate decreased and larvae took longer to emerge and did so at a smaller size. Larval survival to metamorphosis increased with salinity up to 8 g/L (12.45 mS/cm) and then declined at 16 g/L. Exposure to salinity in the larval stage had no effect across metamorphosis on both the adult thorax muscle mass and flight performance, and the investment in immune function. Increasing salinity in the larval stage also had no effect on the energy available but increased the energy consumption in the adult stage, resulting in a lower net energy budget. These negative sublethal effects of increasing salinity hence bridged metamorphosis and contrasted with the mortality data, suggesting that the higher mortality at the low salinity levels selected for larvae with the best body condition. Our results highlight the importance of taking into account other life-history and physiological traits, besides mortality, ideally across different life stages, to better understand and predict consequences of increasing salinization on freshwater insects.

### *Introduction*

Water salinity is a major determinant of habitat suitability and a key driver of species distributions in aquatic insects (e.g. Silberbush et al. 2005; Carver et al. 2009; Carbonell et al. 2012; Galván et al. 2016). Water salinity levels are increasing worldwide because of human activities such as road salinization, industrialisation and urbanisation, agriculture, and longer drought periods under global warming, and may thereby negatively affect an increasing number of aquatic species (Cañedo-Argüelles et al. 2019). This is resulting in a renewed interest in the impact of current and future salinity levels on species of conservation concern (e.g. Beatty et al. 2011; Herbst et al. 2013). To gain insight into the role of salinity levels in driving species distribution patterns and their conservation, it is imperative to understand the tolerance of a species to a gradient of salinity levels (Herbst et al. 2013, Spomer et al. 2015; Tab. 2.1).

Recent studies show that species preferring waters with high salinity may do so for two very different reasons (Arribas et al. 2019). On the one hand, species can be physiologically adapted to high salinities and consequently have a higher fitness under these abiotic conditions (e.g. Browne & Wanigasekera 2000). Species occupying saline waters indeed often show specific osmoregulatory adaptations (Evans 2019). On the other hand, species may rely on high saline water bodies to escape biotic interactions such as predation, parasitism or competition as the combination of direct and indirect effects of salinity drives the composition of communities (e.g. Telesh et al. 2013, Bray et al. 2018). Under the latter scenario, species occupying saline water bodies may do so even at a fitness cost of dealing with high salinity levels (Céspedes et al. 2013). The emerging insight is that species of aquatic insects inhabiting saline waters are generalists, with a predominant pattern of high survival also at low salinity levels, where their fitness tends to be similar or even higher than in saline waters (Arribas et al. 2019).

Despite the importance of disentangling these two reasons for why species prefer high salinity levels, some knowledge gaps remain. Current knowledge on the costs of high salinity levels is mainly limited to fitness effects measured in the aquatic life stages (Arribas et al. 2019). For the many semi-aquatic organisms with an aquatic larval stage and terrestrial adult stage, stressors imposed in the larval stage may have no effects during the larval stage, yet become detectable during or after metamorphosis (e.g. Debecker et al. 2017). Furthermore, studies evaluating costs of exposure to salinity mainly focused on life-history (Stoks et al. 2014), while costs of a stressor may only become detectable when looking at physiology (Karl et al. 2011; Arribas et al. 2019). Given their link with fitness, particularly relevant physiological variables to quantify response to salinity are the net energy budget and the investment in immune function. The few existing studies on this topic demonstrated that high salinity levels reduce the net energy budget (Nayar & Sauerman 1970; Verslycke & Janssen 2002).



Temporary water bodies, that are of special conservation value (Della Bella et al. 2005), often face increasing salinity levels in the drying phase. Species inhabiting temporary fresh water bodies may therefore not only need various adaptations to drought, but also to increasing salinity levels (Williams 1996). On the one hand, the increasing salinity may be used as a cue for pond drying, leading to an acceleration of the development as an adaptive strategy for semi-aquatic animals to metamorphose earlier into terrestrial adults. For example, an exposure to elevated salinity during later development of the tadpoles of *Anaxyrus terrestris* lead to earlier metamorphosis (Welch et al. 2019). Also fixed high salinity levels may have this effect. For example, tadpoles of *Fejervarya limnocharis*, which usually breed in temporary pools but also in brackish water pools in coastal areas, respond to fixed high salinity levels by an earlier metamorphosis (Wu & Kam 2009). Yet, evidence for life-history acceleration during seasonal evaporation of temporary habitats in aquatic insects is limited to mosquito larvae (Schäfer & Lundström 2006), while no support has been found in damselfly larvae when using cues as decreasing water level and therefore increasing larval density and water temperature (De Block & Stoks 2005b). On the other hand, salinity may act as a stressor altering metabolic functions, slowing down growth and development, and eventually resulting in mortality (e.g. Kefford et al. 2003; Mangahas et al. 2019).

In this study, we assessed the effect of salinity on embryonic and larval life-history traits, and on carry-over effects on adult fitness-related traits in the damselfly *Lestes macrostigma* (Eversmann, 1836) (Odonata: Lestidae). This species inhabits temporary, often coastal, brackish water bodies and has a very patchy geographic distribution ranging from Portugal to Siberia (Boudot & Raab 2015). The species avoids fresh water bodies where successful reproduction is anecdotic, at least in the western part of its distribution area (Kosterin 2015; Berquier & Andrei-Ruiz 2019). Adults can emerge successfully from waters with salinity up to ca. 22 g/L matching a conductivity up to 33.3 mS/cm (Lambret et al. 2009). Yet, to what extent the species performs best at high salinity in terms of embryonic and larval life-history, and adult fitness-related performance and physiology, remains to be tested.

Following the emerging pattern identified by Arribas et al. (2019), two opposite predictions could be made. On the one hand, assuming the species is a high-salinity specialist, we predicted higher survival and better performance at higher salinity levels. In addition, high salinity levels may reflect conditions of a pond in an advanced state of drying and therefore trigger a faster growth and/or development. On the other hand, assuming the species is a salinity generalist, we expected similar survival and performance at low salinity and high salinity levels. We tested these hypotheses by carrying out two laboratory experiments. In a first experiment, we exposed eggs to three salinity levels and tested for effects on hatching success and hatching time. Afterwards, we monitored delayed effects in terms of larval development until adult emergence at one salinity level. In a second

experiment, we kept eggs at low salinity, but exposed larvae until adult emergence to one of four salinity levels and tested for effects on larval life-history traits, and carry-over effects on the flight performance and fitness-related physiological traits in the adult stage.

## *Methods*

### *Study species*

Female *L. macrostigma* lay eggs during late spring inside the shoots of several plant species above the water surface (Matushkina & Lambret 2011; Lambret et al. 2018). Individuals remain in the egg stage during the drought season and the following winter, protected by the oviposition plants they were laid in (Lambret et al. 2017). While shoots with eggs are out of water during the drought season, these are flooded in autumn or winter, and water is necessary to trigger hatching (Lambret et al. 2018). Larvae begin to hatch in mid-March and 50% have hatched by the end of March (Lambret et al. 2017). At this time, the salinity of ponds inhabited by *L. macrostigma* ranges from ca. 1.3 to 10 g/L (conductivity range 2.3–15.6 mS/cm, n = 24 ponds studied in 2014 across the Camargue, southern France, Lambret unpubl.). The salinity levels increase during the larval growth period because of evaporation of pond water. Adults start to emerge during the first two weeks of May (Lambret 2010). At this time, salinity ranges from 1.3 to 16.9 g/L (conductivity range 2.5–25.3 mS/cm, n = 21 ponds studied in 2014, Lambret unpubl.). The species is univoltine and larval growth is fast, like many other *Lestes* species inhabiting temporary ponds (e.g. Stoks & McPeck 2003b; Schiel & Buchwald 2015b).

### *Egg sampling and embryonic development*

For the first experiment, 31 shoots of *Juncus maritimus* containing 1,342 eggs were collected during June 2016 at a water body of the Marais du Vigueirat National Nature Reserve (43°29'27"N | 04°48'22"E, southern France) following the procedure of Lambret et al. (2018). Each shoot contained  $43.3 \pm 20.3$  eggs (mean  $\pm$  SD). As a female lays  $9.4 \pm 9.5$  eggs inside one shoot (Lambret et al. 2015b, Lambret unpubl.), it is highly probable that each of the 31 shoots contained eggs laid by several females, and that we have sampled offspring of more than 100 females. Matching natural conditions, eggs remained in their shoot during the entire experiment. During summer–autumn, shoots were kept dry indoors under the natural photoperiod but cooler than outside at ca. 25 °C in order to reduce desiccation risk and therefore egg mortality (see Lambret et al. 2018). Flooding of temporary ponds was mimicked by submerging shoots individually on 16 November 2016 in 4 × 10 × 16 cm plastic boxes filled with 0.2 L of tap water (Fig. 2.1a), and kept outdoors at the Tour du Valat Research Institute until

7 February 2017. During this period, the mean air temperature was 7.8 °C (Météo France, meteorological station of 'Arles-Valat', n°13004003, 43°30'36"N | 4°41'38"E, 2 km from the study site). From 7 February 2017 until hatching, the shoots with eggs were assigned to three different salinity levels and eggs were distributed as follows: 361 eggs at 2 g/L (11 shoots), 382 eggs at 5.5 g/L (10 shoots) and 599 eggs at 9.5 g/L (10 shoots). These salinity levels correspond to those observed in situ (see *Study species*). Although various ions may contribute to water salinity and impact osmoregulation (e.g. Herbst et al. 1988), sodium chloride (NaCl) is dominant in the salt composition of the water bodies inhabited by *L. macrostigma* (Aguesse 1961). Hence, the salt solutions were obtained by dissolving into distilled water natural sea salt produced in the Camargue (Salins du Midi Company) by fully evaporating sea water (97% NaCl). Between 7 February and 10 March 2017, shoots were kept in a fridge at ca. 3 °C to prevent hatching (Fig. 2.1b). To trigger hatching, shoots were set in an incubator (JP Selecta® EC500GL, Abrera, Spain) at progressively increasing temperatures and photoperiods to finally mimic April natural pond conditions: 10 °C and 10|14 Light|Dark (10–16 March); 15 °C and 11.5|12.5 L|D (17–23 March); and 20 °C and 13|11 L|D (24 March onwards).

For the second experiment, seven shoots of *Bolboschoenus maritimus* and eight shoots of *Schoenoplectus tabernaemontani* containing egg clutches were collected on 8 July 2018 in a temporary water body within the Tour du Valat Regional Nature Reserve (43°29'01"N | 40°40'35"E). Shoots were first kept at room conditions (same as above) and then transferred on 31 July 2018 to an incubator at 15 °C and a 12|12 L|D photoperiod to mimic autumn conditions. Shoots were submerged individually on 13 November 2018 in plastic boxes (same as above) filled with 0.2 L of tap water; incubator conditions were adjusted to 10 °C and a 9|15 L|D photoperiod. To mimic winter conditions, plastic boxes were placed in a fridge at 2–4 °C and total darkness on 30 November 2018. To trigger hatching, plastic boxes were set in an unheated room at 11–18 °C (mean = 15 °C, n = 247) on 18 February 2019 with a natural photoperiod (i.e. ca. 11|13 L|D photoperiod) and then, from 25 February 2019 onwards in an incubator at 19 °C and a 12|12 L|D photoperiod.

Table 2.1 – Physiochemical characteristics of the tap water, distilled water and salt solutions used during experiments 1 and 2.

Parameters	Tap water	Distilled water	Salt solutions						
Salinity (g/L)	0.4	0	0.5	2	4	5.5	8	9.5	16
Conductivity at 20 °C (mS/cm)	0.78	0.05	1.01	3.45	6.53	8.56	12.45	14.52	23.35
pH	6.47	7	6.60	6.54	6.44	6.51	6.44	6.49	6.47

### *Rearing of larvae and treatments*

In experiment 1, we continued rearing larvae that hatched from the boxes with 2 g/L salinity ( $n = 142$ ) in the incubator at 2 g/L salinity at 20 °C with a 13|11 L|D photoperiod. Larvae that hatched from the boxes at other salinity levels were released in the field. From 22 March, boxes were checked every two days for the presence of hatched larvae. To allow individual monitoring in the larval stage, hatchlings were placed separately in transparent plastic cups (4 cm high  $\times$  4 cm in diameter) in the incubator (Fig. 2.1c). Larvae were fed ad libitum every two days with Cladocera (Fig. 2.1c) caught in a nearby pond. We gradually increased prey size to match the increase in damselfly size. During feeding, the survival of larvae and the presence of moult were checked; moults, faeces and prey remains were removed with a pipette. The water level was kept constant by adding the 2 g/L salt solution.

In experiment 2, we exposed larvae to following salinity levels: 69 larvae at 0.5 g/L, 75 at 4 g/L, 64 at 8 g/L and 104 at 16 g/L; the salt solutions were prepared as described above. Hatching started on 26 February. Hatchlings were assigned to the four salinity levels as evenly as possible with regard to the shoots they were coming from. Following hatching, larvae were placed individually in white plastic cups (same dimensions as above) and monitored daily. They were fed each day in the morning (9:30 AM) ad libitum with nauplii of the brine shrimp *Artemia salina*, a prey tolerating a larger salinity range than Cladocera. To avoid salinity variation in the cups, nauplii were first rinsed with water matching the salinity level of the cups. These salinity levels are below natural levels experienced by nauplii. Hence, the effect salinity levels may have on nauplii is a confounding factor acting on *L. macrostigma* larvae. In a preliminary experiment, we assessed the survival of the nauplii at these four salinity levels. After 15 h, the survival rate of nauplii was high at 16, 8 and 4 g/L (respectively: 98%,  $n = 176$ ; 96%,  $n = 206$ ; 98%,  $n = 289$ ) but decreased to 68% ( $n = 193$ ) at 0.5 g/L. To avoid confounding water salinity level and food availability, we fed the larvae reared at 0.5 g/L twice a day (morning and around noon). Moreover, we renewed the medium of all cups daily and thereby removed all remaining nauplii from cups at 4:30 PM.

During each experiment, the position of the cups on the shelves and of the shelves in the incubator was randomized several times per week. When larvae reached F-0 (the final larval stage), they were transferred to a bigger white plastic cup (9 cm high  $\times$  4 cm bottom diameter  $\times$  6 cm top diameter) containing a vertical plastic grid to allow adults to emerge. These cups were covered with a 2 mm mesh tissue to prevent adults to leave their cup (Fig. 2.1d). From the date of first emergence the presence of adults was checked daily.

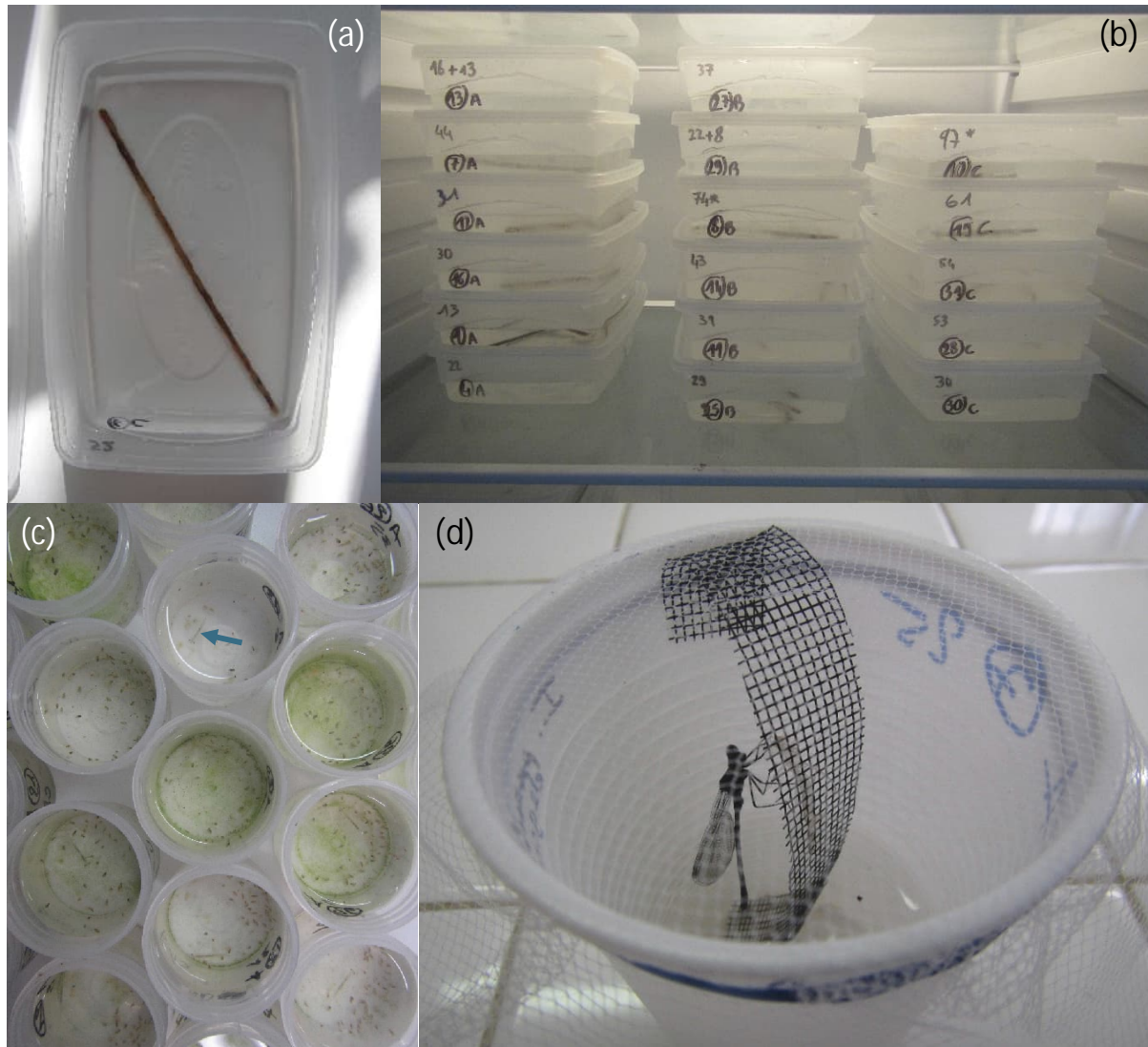


Figure 2.1 – Experimental set-up with: (a) shoots containing *Lestes macrostigma* eggs placed in individual plastic boxes filled with water; (b) shoots kept in a fridge at ca. 3 °C to prevent hatching; (c) each *L. macrostigma* (arrow) larva set in individual transparent plastic cups with Cladocera as prey; (d) plastic cup with vertical plastic grid to allow adults to emerge and covered with a mesh to prevent them to leave their cup.

*Measurement of life-history traits*

For each experiment, we recorded the dates of hatching, and of death or emergence. This allowed us to calculate both lifespan in larvae which died before emergence, and development time in larvae which emerged. Larval lifespan and development time were expressed in number of days between hatching date and date of death or emergence, respectively. Hatching dates were expressed as Julian dates, with day one being 1 January. We coded emergence success as 0 for larvae which died before reaching the adult stage, and 1 for larvae which emerged. After emergence, we recorded the sex.

In experiment 1, we additionally recorded the number of moults between the second larval stage (after the prolarval stage i.e. when the larva has hatched out of its egg-shell but has not yet casted its embryonic cuticle; see Corbet 2002) and adult emergence. In experiment 2, to obtain an estimate of growth rate, we measured the head width of adults after emergence ( $n = 55$ ) and on two subsets of larvae at two moments of larval development: just after hatching ( $n = 33$ ) and at death during larval development ( $n = 130$ ). We measured the head width to the nearest 0.02 mm using a micrometer eyepiece under a stereo microscope (Zeiss® Stemi 2000, Oberkochen, Germany) at a magnification of 10.6 ×. For those larvae that survived at least seven days, we calculated larval growth rate as:

$$\text{growth rate} = \left| \frac{\ln(\text{head width at death or emergence}) - \ln(\text{head width at hatching})}{\text{date of death or emergence} - \text{date of hatching}} \right|$$

(Schiel & Buchwald 2015a). The mean head width at hatching based on the measured subset was 0.48 mm (min = 0.40 mm, max = 0.57 mm).

We categorized the metamorphosis success as follows: 0 for animals that did not fully cast their larval skin and died during metamorphosis; 1 for animals that fully casted their larval skin but had at least one not fully extended wing; and 2 for animals that fully casted their larval skin with four normally extended wings. One day after metamorphosis, we categorized the flight ability of the adults as: 0, not able to fly; 1, able to fly. To this end, we placed each adult at the bottom of a vertical Plexiglass tube (30 cm in diameter × 160 cm high). We gently tapped the abdomen when the individual did not spontaneously fly after 5 min. Then, we measured the wet mass of the adults with an electrobalance (Mettler Toledo® AG245, Ohio, USA) to the nearest 0.1 mg. All adults were then stored separately in paper envelopes in a freezer at –80 °C until physiological analyses (see below).

*Measurement of physiological traits in the adults*

All physiological measurements were done using spectrophotometry following the assays described in detail in Van Dievel et al. (2017). First, we separated the thorax from the rest of the body. The thorax

was crushed, homogenized and dissolved in 150  $\mu\text{L}$  of phosphate buffered saline (50 mM/L, pH 7.4; hereafter PBS). The solution was centrifuged for 8 min at 13,000 rpm and 4 °C. On this thorax homogenate, we measured the protein concentration. In adult odonates, the flight muscles make up most of the thorax and the thorax protein content can therefore be used as a proxy for flight muscle mass (e.g. Therry et al. 2014). 1  $\mu\text{L}$  of the thorax homogenate was mixed with 160  $\mu\text{L}$  of ultra-pure water (purification with Milli-Q® system, Merck Millipore, Burlington, USA; hereafter UPW) and 40  $\mu\text{L}$  of protein assay dye reagent (Bio-Rad®, Hercules, USA). The solution was incubated for 5 min at 25 °C and the absorbance read at 595 nm on four technical replicates per biological sample (i.e. per adult). The mean absorbance per biological sample was converted to a protein concentration using a standard curve with albumin. Based on this concentration, the thorax muscle mass was calculated. Flight performance in odonates increases as the ratio of flight muscle mass to the total mass increases (Marden 2000). Hence, we divided the thorax muscle mass by the wet body mass and expressed this ratio as  $\mu\text{g}$  per mg body mass.

Afterwards, the rest of the adult was added to the thorax homogenate, crushed, and the volume was adjusted with PBS [final volume ( $\mu\text{L}$ ) =  $10 \times$  wet mass (mg)]. The solution was homogenized and centrifuged again as above. All other analyses were done on this total homogenate. We determined the two parameters related to the cellular energy allocation (CEA): the available energy reserves ( $E_a$ ) and the energy consumption ( $E_c$ ). To estimate  $E_a$ , the total protein, fat and sugar contents were measured (De Coen & Janssen 2003). The total protein content was quantified as above. The total fat content was determined by mixing 8  $\mu\text{L}$  of the total homogenate with 56  $\mu\text{L}$  of concentrated sulfuric acid in a fat-free glass tube. The solution was incubated for 20 min at 150 °C. After cooling at room temperature, 64  $\mu\text{L}$  of UPW were added. Per biological sample, we read the absorbance at 340 nm on 30  $\mu\text{L}$  triplicates per biological sample. A standard curve made with glyceryl tripalmitate was used to convert the mean absorbance per biological sample into fat content. To quantify the total sugar concentration, 5  $\mu\text{L}$  of total homogenate were mixed with 13  $\mu\text{L}$  of PBS and 2  $\mu\text{L}$  of amyloglucosidase solution (0,1 U/ $\mu\text{L}$ , Sigma-Aldrich® A7420, Saint-Louis, USA). The solution was incubated for 30 min at 37 °C. Then, 40  $\mu\text{L}$  of glucose reagent (Sigma® G3293) were added and the mixture incubated for 20 min at 30 °C. The absorbance was measured at 340 nm in triplicate, and converted in total sugar content using a calibration curve with glucose. The body contents of the three storage molecules were expressed in  $\mu\text{g}$  per mg wet body mass. We calculated  $E_a$  in Joule (J) by converting protein, fat and sugar contents into energetic equivalents using the following combustion energy values: 24 J/mg for proteins, 39.5 J/mg for fat and 17.5 J/mg for sugars (De Coen & Janssen 2003).

As a measure of energy consumption, hence  $E_c$ , we measured the activity of the Electron Transport System (ETS) (De Coen & Janssen 2003). In the reaction of the assay, Iodonitrotetrazolium

chloride (hereafter INT) replaces  $O_2$  as electron acceptor in the mitochondria; during this reaction formazan (red coloured) is formed. The INT reagent was prepared by mixing 40.6 mg of p-iodonitrotetrazolium violet (Sigma® I8377) in 10 mL of distilled water. The reaction mixture was prepared in two steps. A first solution was made by mixing 8.083 g of Tris [tris(hydroxymethyl)aminomethane] and 1.366 mL of Triton X-100 in 500 mL of distilled water, with pH adjusted to 8.5 with hydrochloric acid. On the day of analysis, 11.9 mg of reduced nicotinamide adenine dinucleotide (NADH) and 2.1 mg of reduced nicotinamide adenine dinucleotide phosphate (NADPH) were mixed in 10 mL of the first solution. 5  $\mu$ L of the total homogenate were mixed in 15  $\mu$ L of this reaction mixture. The reaction started by adding 10  $\mu$ L of INT reagent. The kinetic run was monitored by measuring the absorbance at 490 nm during a 5 min period at 20 °C. We computed the mean reaction slope in triplicate per biological sample. We determined the amount of consumed  $O_2$  during ETS activity given that 2 mol of formazan formed corresponds to 1 mol of  $O_2$  (De Coen & Janssen 2003).  $E_c$  (in J) was calculated by transforming this amount of  $O_2$  based on the specific oxyenthalpic equivalents of 480,000 J/mol  $O_2$  for an average protein, fat and sugar mixture. CEA was then calculated as  $E_a/E_c$  (Verheyen & Stoks 2020).

As a measure of investment in immune function and body condition, we measured the activity of the enzyme phenoloxidase (PO) (González-Santoyo & Córdoba-Aguilar 2012). PO plays a key role in the production of several molecules, such as melanin and cytotoxic quinones, which are involved in the reaction against pathogens. 10  $\mu$ L of the total homogenate were mixed with 10  $\mu$ L of PBS and 5  $\mu$ L of chymotrypsin. The solution was incubated for 5 min at room temperature. The reaction started by adding 15  $\mu$ L of L-Dopa substrate (19.7 mg of dihydroxyphenyl-L-alanine in 10 mL of cacodylate buffer, i.e. 10 mM). The kinetic run was monitored by reading the absorbance at 490 nm during a 45 min period at 30 °C. We computed the mean reaction slope in triplicate per biological sample. PO activity was expressed in units with 1 unit representing 1 mmol dopachrome formed per minute and per mg protein.

### *Statistical analyses*

In experiment 1, we used Generalized Linear Mixed Models (GLMMs) to test the effect of salinity level (fixed factor, as categorical variable) on hatching success and hatching date, and to test the effect of sex on larval development. We used a nested design and included 'shoot' as a random effect to take into account that several individuals had shared the same shoot in the egg stage. In experiment 2, we used GLMs to test the effects of salinity level (same as above). Metamorphosis success (0, 1 or 2) was analysed using an ordered logit regression. We could not use GLMMs including 'shoot' as random



effect because the number of larvae per shoot was too low to get confident estimates. Note that a possible effect of shoot was minimized by assigning hatchlings evenly to the four salinity treatments. When testing the effect of salinity on growth rate, we first assessed whether this effect differed between larvae that reached emergence and those that died before. We therefore used a GLM with salinity levels, development type (i.e. complete or incomplete), and their interaction, as fixed factors. Development type had a significant effect on growth rate ( $t = -3.457$ ,  $p < 0.001$ ). However, the interaction was non-significant ( $F_{(178, 180)} = 0.322$ ,  $p = 0.725$ ), indicating that the effect of salinity on growth rate was similar for larvae of both development types. Yet, instantaneous growth rate may also vary with the age of larvae in insects (Tammaru & Esperk 2007). Hence, when testing the effect of salinity on the growth rate of all larvae, we used a GLMM with larval age as random effect.

GLMs and GLMMs do not compute mean values or frequencies but estimate them from the data (Bolker 2008). Results are therefore given as estimated frequencies or mean values with their 95% confidence intervals (i.e.  $\pm 1.96 \times \text{SE}$ , here after CI). Significance levels were assessed with a variance analysis for GLMs and a likelihood ratio test for GLMMs. P-values lower than 0.05 were considered as statistically significant. In cases where  $0.05 < p < 0.1$ , to assess whether low significance could be due to a small sample size, we added a power analysis and provided  $\beta$  (type II error), the power of the analysis being  $1 - \beta$  (Quinn & Keough 2002).

Emergence success (0 or 1) was analysed using a binomial error structure (Zuur et al. 2009). We log transformed hatching and emergence dates, development time, Ec and CEA, and square-root transformed lifespan and PO activity to normalise the data. Normalised data, size at emergence, growth rate, and Ea were analysed using a Gaussian error. We checked Gaussian model assumptions (normality, heteroscedasticity and independence of residuals) following Zuur et al. (2009). All analyses were performed in R 3.6.3 (R Core Team 2020), using packages 'lme4' (Bates et al. 2019), 'lmerTest' (Kuznetsova et al. 2020), 'lmerTest' (Hothorn et al. 2020), 'ggeffects' (Lüdtke & Aust 2020), 'MASS' (Ripley 2020) and 'simr' (Green & MacLeod 2019); figures were made using package 'ggplot2' (Wickham et al. 2020).

## Results

### *Experiment 1: Effect of salinity on egg hatching, and larval development at 2 g/L*

The model estimated that 73%, 95% CI = [53%, 87%], of the eggs hatched. Hatching success remained constant between 2 and 5.5 g/L but then decreased at 9.5 g/L (Fig. 2.2). However, we did not detect a significant effect of salinity on hatching success ( $\chi^2_2 = 2.120$ ,  $p = 0.346$ ). The mean hatching date was 6 April 2017 [4 April, 9 April], i.e. 27 days after transferring the eggs at 20 °C into the incubator (10 March

2017). Salinity had no significant effect on hatching date ( $\chi^2_2 = 2.040$ ,  $p = 0.360$ ) which slightly advanced from 8 April [5 April, 13 April] at 2 g/L to 5 April [1 April, 8 April] at 9.5 g/L.

Among the 142 larvae reared at 2 g/L, 73% [59.7%, 83.1%] reached the final larval instar (F0) and this occurred on average after 45.4 days [44.1, 46.6]. Of the 72 larvae for which we assessed the number of moults to reach F-0, 16 larvae (22.2%) showed six moults, 48 larvae (66.7%) seven moults, and eight larvae (11.1%) eight moults. Emergence success of the 142 larvae was 64.7% [50.1%, 76.9%]. The total development time from egg to adult did not differ significantly between sexes (estimate =  $0.009 \pm 0.030$ ;  $t = 0.596$ ,  $p = 0.557$ ) and took on average 65.3 days [64.6, 66]. The lifespan of larvae which died before emergence was on average 39.6 days [33.7, 45.5].

### *Experiment 2: Effect of salinity on larval life-history and adult traits*

The increase in time the larvae needed to emerge with increasing salinity was nearly significant ( $F_{(2, 309)} = 3.083$ ,  $p = 0.054$ ,  $\beta = 0.696$ ; Fig. 2.3a): this time was significantly higher at 8 g/L than at 4 g/L ( $t = 2.394$ ,  $p = 0.020$ ) but other post-hoc comparisons were not significant. Considering all larvae which survived at least one week, growth rate at 16 g/L was significantly lower than at lower salinity levels ( $\chi^2_3 = 40.233$ ,  $p < 0.001$ ; Fig. 2.3b).

In total, 58 adults emerged: nine at 0.5 g/L, 20 at 4 g/L, 29 at 8 g/L but none at 16 g/L. Emergence success increased significantly with salinity up to 8 g/L but then declined toward salinity 16 g/L ( $F_{(3, 308)} = 23.692$ ,  $p < 0.001$ ; Fig. 2.4a). For those larvae which died before emergence (i.e. before reaching the adult stage), lifespan was significantly influenced by salinity ( $F_{(3, 251)} = 17.639$ ,  $p < 0.001$ ), following the same pattern as emergence success (Fig. 2.4b). Among the 58 adults that emerged, 49 adults did so successfully: eight at 0.5 g/L, 18 at 4 g/L and 23 at 8 g/L. Metamorphosis success decreased with increasing salinity (Tab. 2.2) but we did not detect the effect of salinity as significant ( $\chi^2_2 = 0.888$ ,  $p = 0.642$ ). Size at emergence decreased nearly significantly with increasing salinity ( $F_{(2, 52)} = 2.790$ ,  $p = 0.071$ ,  $\beta = 0.442$ ; Fig. 2.5a), because of the smaller size at 8 g/L compared to 4 g/L ( $t = -1.818$ ,  $p = 0.075$ ) and especially to 0.5 g/L ( $t = -1.973$ ,  $p = 0.054$ ). In contrast, considering only the adults that fully casted their exuvia at emergence, and therefore that fully extruded their haemolymph, wet mass at emergence was not affected by salinity ( $F_{(2, 43)} = 1.806$ ,  $p = 0.176$ ; Fig. 2.5b). We did not detect an effect of salinity on flight ability ( $\chi^2_2 = 0.636$ ,  $p = 0.727$ ), neither on the thorax muscle mass ratio ( $F_{(2, 48)} = 1.226$ ,  $p = 0.302$ ) (Tab. 2.2).

Table 2.2 – Effect of salinity on adult traits of *Lestes macrostigma*. Metamorphosis success is given as the ratio of adults that fully emerged (category 2). Other frequencies and means are GLM estimated values with their 95% confidence intervals.

	Salinity (g/L)		
	0.50	4	8
Metamorphosis success	0.77 [0.42, 0.94]	0.67 [0.45, 0.83]	0.61 [0.42, 0.77]
Flight ability	0.83 [0.37, 0.98]	0.67 [0.38, 0.87]	0.69 [0.43, 0.86]
Thorax protein ratio (µg/mg)	12.5 [10.8, 14.1]	11.8 [10.6, 13]	13 [12, 14.1] <sup>ns</sup>
Protein content (µg/mg)	22.8 [19.3, 26.3]	24.8 [22.3, 27.2]	25.2 [23.1, 27.3]
Fat content (µg/mg)	13.3 [10.7, 15.9]	13.4 [11.6, 15.3]	11.6 [10, 13.2]
Sugar content (µg/mg)	7.5 [5.6, 9.3]	6.4 [5.1, 7.7]	5.8 [4.7, 7]
Energy available (J)	55.8 [45.1, 66.6]	63.2 [55.6, 70.8]	54 [47.4, 60.5]
Energy consumption (kJ)	26.1 [22, 31.3]	28.3 [24.8, 31.9]	31.3 [28, 34.5]

We did not detect significant effects of increasing salinity levels imposed during the larval stage on the protein, fat and sugar contents of the adults (protein:  $F_{(2, 48)} = 0.680$ ,  $p = 0.511$ ; fat:  $F_{(2, 48)} = 1.338$ ,  $p = 0.272$ ; sugar:  $F_{(2, 48)} = 1.174$ ,  $p = 0.318$ ; Tab. 2.2). The integrated measure, the amount of energy available (Ea) was also not affected by the salinity level ( $F_{(2, 48)} = 1.785$ ,  $p = 0.179$ ; Tab. 2.2). However, adults had a slightly higher energy consumption (Ec) with increasing salinity (Tab. 2.2) although this was not significant ( $F_{(2, 48)} = 1.668$ ,  $p = 0.199$ ). Further, adults had a nearly significantly lower net energy budget (CEA) at higher salinity level ( $F_{(2, 48)} = 2.435$ ,  $p = 0.098$ ,  $\beta = 0.521$ ; Fig. 2.6a). This was due to the CEA at 8 g/L being nearly significantly lower than that at 4 g/L ( $t = 2.005$ ,  $p = 0.051$ ). The phenoloxidase activity, i.e. investment in immune function, was lower at 4 g/L and 8 g/L than at 0.5 g/L but this was not significant ( $F_{(2, 48)} = 1.241$ ,  $p = 0.298$ ; Fig. 2.6b).

Figure 2.2 – Effect of salinity on egg hatching success of *Lestes macrostigma*. The squares and bars indicate the frequencies and 95% confidence intervals estimated by the generalized linear mixed model.

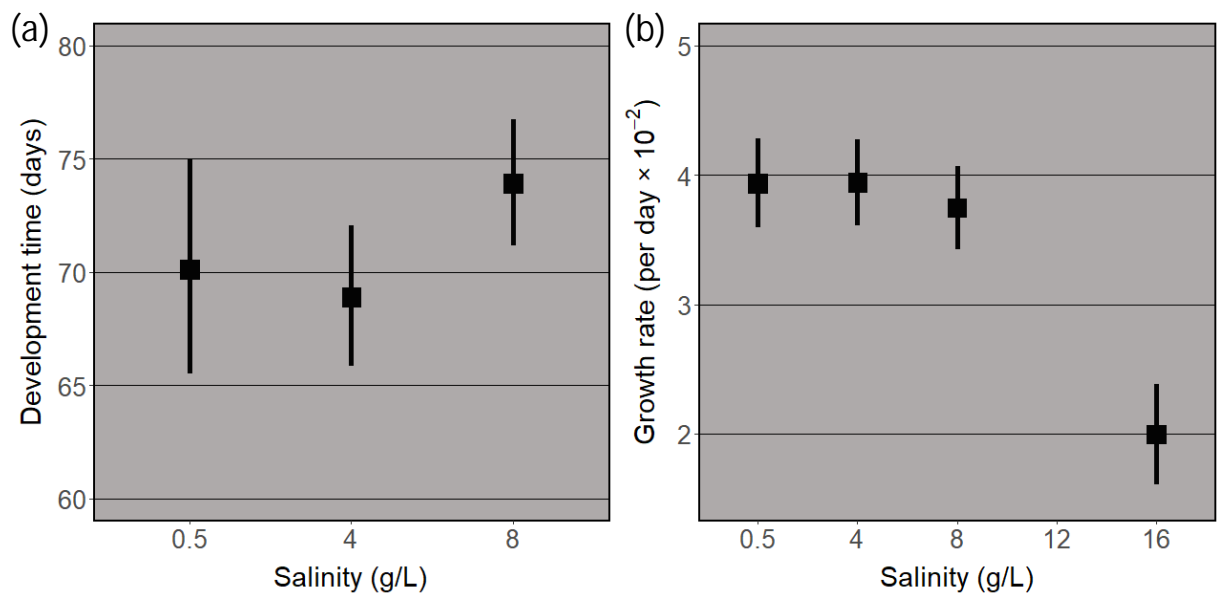
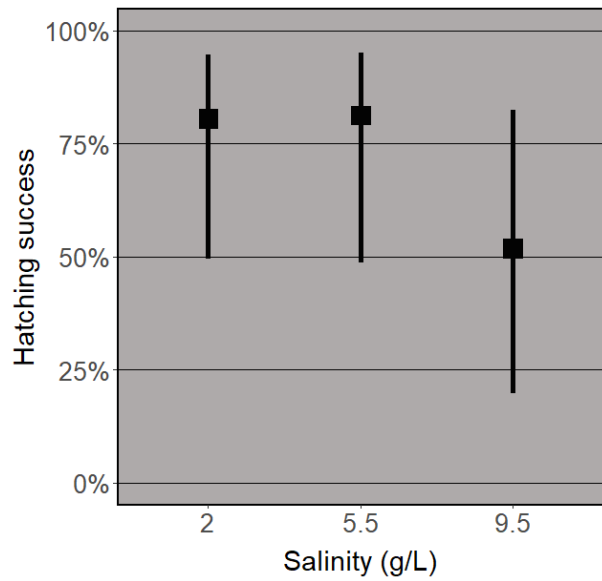


Figure 2.3 – Effect of salinity on development time of larvae of *Lestes macrostigma* that emerged (a) and on growth rate of larvae that were at least seven day old (b). The squares and bars indicate the frequencies and 95% confidence intervals estimated by the generalized linear models.

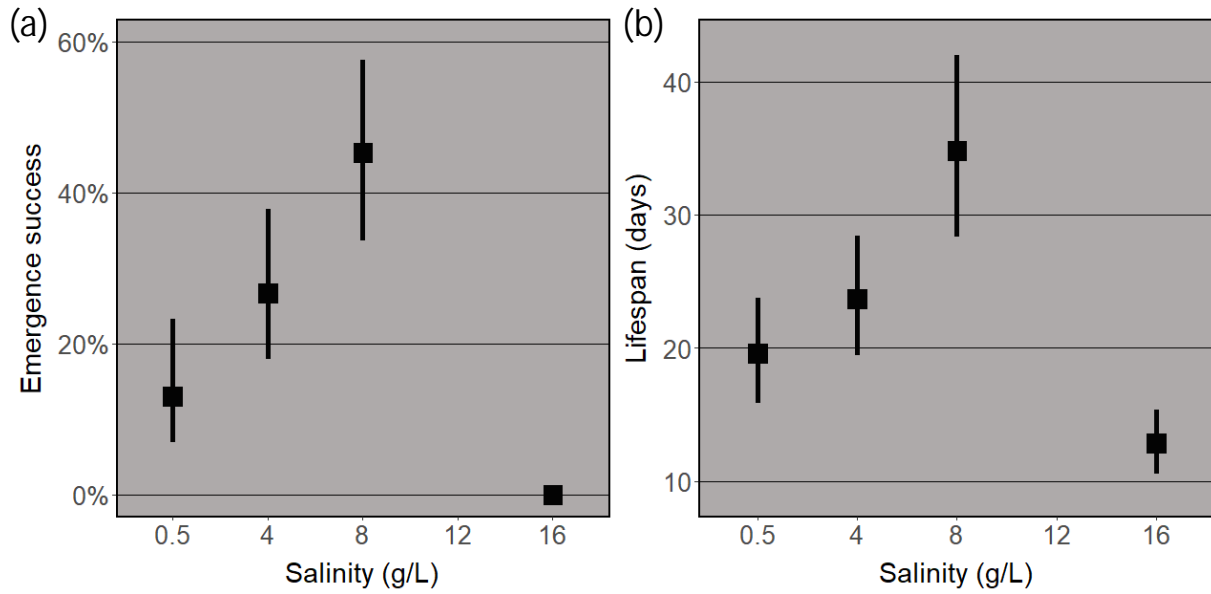


Figure 2.4 – Effect of salinity on *Lestes macrostigma* adult emergence success (a) and on lifespan of larvae that died before emergence (b). The squares and bars indicate the frequencies and 95% confidence intervals estimated by the generalized linear models.

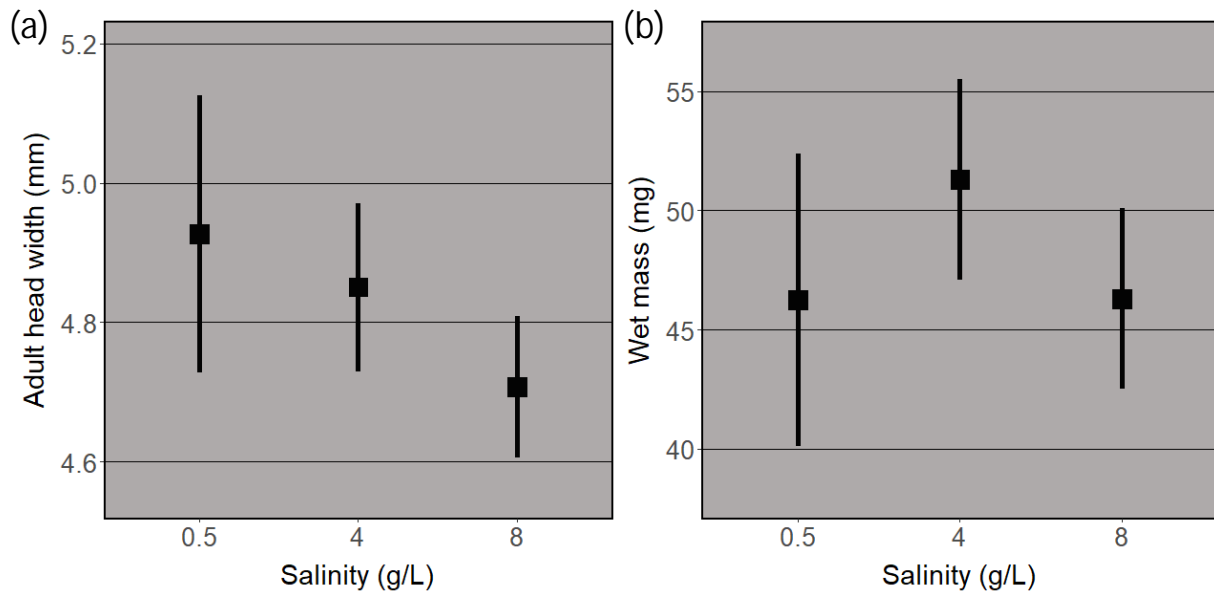


Figure 2.5 – Effect of salinity on size (a) and wet mass (b) at emergence of *Lestes macrostigma*. The squares and bars indicate the frequencies and 95% confidence intervals estimated by the generalized linear models.

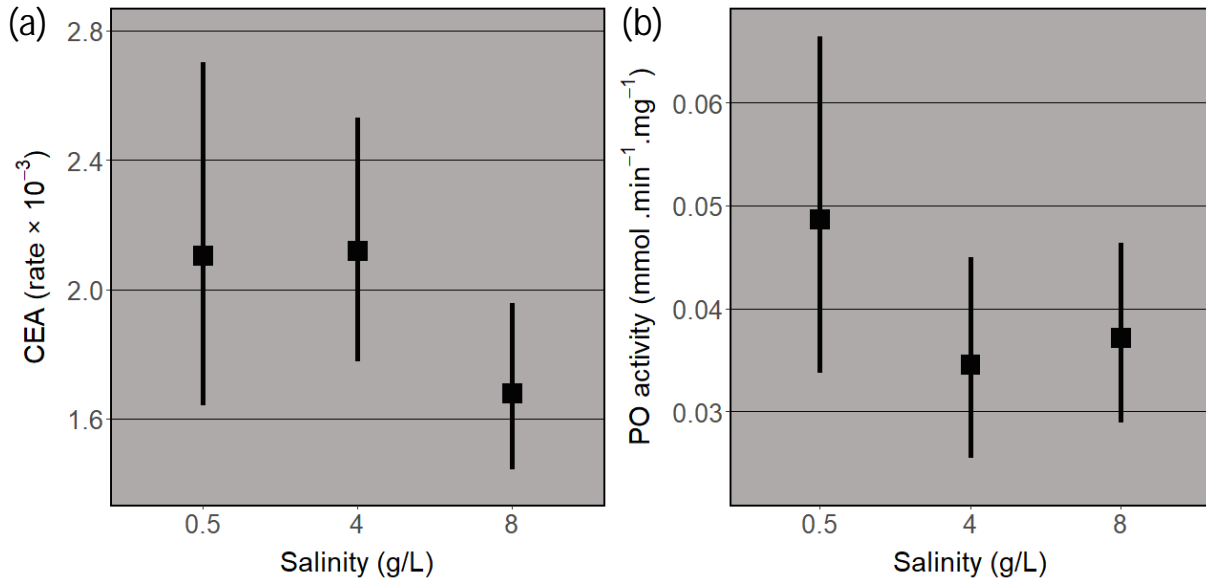


Figure 2.6 – Effect of salinity on cellular energy allocation (a) and phenoloxidase activity (b) of *Lestes macrostigma*. The squares and bars indicate the frequencies and 95% confidence intervals estimated by the generalized linear models.

### Discussion

The results of experiment 1 suggest that under natural conditions of shoot protection the eggs of the saline water species *L. macrostigma* can cope with a wide salinity range. During the egg stage, *L. macrostigma* survived all salinity levels and increasing salinity had no effect on the post-diapause embryonic development. During hatching, swallowing water of higher salinity to increase the inner egg pressure and break its vitelline membrane (Corbet 2004) is likely to be deleterious for the embryo through toxicity caused by interactive effects of major ions, a decrease in the energy available for other purposes than osmoregulation, or a loss of pH regulation (Scheibener et al. 2016, Kefford 2019). As eggs remained in their shoot, they might have been protected from extreme abiotic conditions (Verberk et al. 2008). Hatching success is known to decrease with increasing salinity in several insects living in freshwater, such as stoneflies or caddisflies (Kefford et al. 2004), but also in, for example, the salt-tolerant boatman *Trichocorixa verticalis* (Carbonell et al. 2016). In the only existing study on damselflies, Iwata & Watanabe (2004) showed that the eggs of the brackish water species *Mortonagrion hirosei* laid in filter paper, i.e. without protection of plant material, did not differ in hatching success from low to intermediate salinity levels, but showed a reduced success at the highest salinity level (20 g/L), which is likely to be experienced following flooding at high sea tide (M. Watanabe pers. comm.). As observed in chironomids and other freshwater ‘invertebrates’ (Kefford et al. 2007), salinity did not change the hatching date.

A key finding of experiment 2 was that adult emergence success, i.e. survival across the entire larval period, increased with increasing salinity up to 8 g/L and then dropped to zero at 16 g/L. Larval survival is a major factor driving the population dynamics and distribution of damselflies (McPeck 2008). The absolute survival rates at the different salinity levels should be considered with caution: compared to our first larval rearing experiment at 2 g/L, the emergence success was lower (21.7% vs 64.7%) across the salinity levels between 0.5 and 8 g/L. It was also lower than the 75% observed by Schiel & Buchwald (2015a), who reared *L. macrostigma* in rainwater. While temperature and photoperiod were similar in both experiments, we used different food. However, nauplii (experiment 2) are commonly used in experiments with damselfly larvae and can even lead to higher growth rates than when feeding with Cladocera (Kefford et al. 2006). Possibly, larval mortality was overall higher in experiment 2 because of the daily manipulation when cleaning the cups, as the stress caused by the mere presence of a potential predator may increase larval mortality and decrease metamorphosis success in odonates (Stoks 2001, McCauley et al. 2011). Nevertheless, our results indicate that larvae of *L. macrostigma* survive better at intermediate than at lower salinity, but not at very high salinity levels (16 g/L). Adults emerging successfully from water bodies with high salinity, up to ca. 28 g/L (Lambret et al. 2009, 2023), are therefore likely to have experienced such high salinity levels only during the latter part of their larval development when the water level of their temporary pond was decreasing. Arribas et al. (2019) identified two types of saline generalists: (1) the 'jack-of-all-trades master of all', performing as well at low and high salinities, and (2) the 'jack-of-all-trades master of none', performing less well at low salinities than at high salinities. The pattern we found in *L. macrostigma* survival is that of a saline generalist of type 2, as has been found in mayflies, chironomids and mosquitoes (Clark et al. 2004, Hassell et al. 2006). This pattern seems to differ with that of other salt-tolerant damselflies: in both *M. hirosei* (Iwata & Watanabe 2004) and *Ischnura heterosticta* (Kefford et al. 2006) survival was high from low to intermediate salinity and then decreased at very high salinity levels, making them saline generalists of type 1.

In contrast with the mortality data, we detected several negative sublethal effects of salinity levels higher than 0.5 g/L during the larval stage and after emergence. Note that this was true despite us being unable to measure physiological traits at 16 g/L as all larvae died before emergence at this highest salinity level. With increasing salinity, *L. macrostigma* developed slower, had a lower growth rate and emerged at a smaller size. These sublethal effects are expected to negatively affect fitness in *Lestes* damselflies, such as lifetime mating success (De Block & Stoks 2005a), especially when there is a risk of pond drying (Stoks & Córdoba-Aguilar 2012). This does not support our hypothesis of an accelerated life-history under salinity stress, and further supports the lack of an adaptive response to the risk of habitat drying in temporary pond *Lestes* damselflies (De Block & Stoks 2005b). Yet, an

accelerated life-history may not occur when larvae are exposed to a salinity stress already during development (Welch et al. 2019) or may be only triggered by a salinity increase during larval development instead of a constant high salinity level. Other studies reported a negative effect of salinity on the growth rate of semi-aquatic insects with aquatic larvae and terrestrial adults. This was explained by a higher energetic investment in osmoregulation, and because of a higher water loss and a reduced foraging activity at higher salinity (e.g. Hassell et al. 2006; Herbst et al. 2013). In contrast, Kefford et al. (2006) reported for *I. heterosticta* that growth rate was enhanced by increasing salinity, although the species is salt tolerant rather than restricted to brackish habitats. Notably, with increasing salinity levels experienced during the larval stage, the adults did not have a lower amount of energy available ( $E_a$ ), yet they did display increased energy consumption ( $E_c$ , measured as ETS activity), resulting in a nearly significant reduction in the net energy budget (CEA). This is in line with the observations that higher salinity lead to an increased respiration in the water boatmen *Sigara selecta* (Carbonell et al. 2017), and a higher sugar consumption ( $E_c$ ) in the estuarine shrimp *Neomysis integer* (Verslycke & Janssen 2002). Yet, Nayar & Sauerman (1970) found that  $E_a$  could also be affected by salinity as higher salinity levels reduced sugar and fat reserves at emergence in the mosquito *Anopheles bradleyi*.

Taken together, *L. macrostigma* appears to be a saline generalist with a broad fundamental niche, which is in line with the emerging understanding of insect species inhabiting saline waters (Arribas et al. 2019). The species shows a broader range of salinity tolerance compared to European congeneric species (e.g. Aguesse 1961; Mielewczyk 1970; Rychła et al. 2011). It is able to emerge from water with salinity levels from 0.5 to 8 g/L, and even higher levels provided that such higher salinity levels are experienced only during a part of the larval period (Lambret et al. 2009, 2023). Considering that *L. macrostigma* mainly inhabits brackish waters, its realized niche is therefore smaller than its fundamental niche, a pattern found in many saline insects (Arribas et al. 2019). Intriguingly, the lethal and sublethal effects of salinity followed two different patterns. On the one hand, larval survival until emergence increased from 0.5 g/L toward 8 g/L. On the other hand, the species performance at the sublethal level decreased with salinity increasing from 0.5 g/L onwards, the typical pattern followed by freshwater instead of saline insects (Arribas et al. 2019). Potentially, lower survival at the low salinity levels selected for the strongest larvae, i.e. these with the best body condition, resulting in better values in the survivors for the sublethal endpoints. Our results highlight the importance to take into account besides mortality, other life-history and physiological traits, ideally across different life-history stages, to better understand and predict future consequences of increasing salinization on freshwater insect distributions.



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### *Author's contributions*

Conceptualization: PL, LJ, RS; Funding acquisition: PL, RS; Project administration: PL; Methodology: PL, LJ, RS; Investigation: PL; Data curation: PL, LJ; Visualization: PL; Writing | original draft: PL, RS; Writing | review & editing: PL, LJ, RS; Supervision: RS.

## Chapter 3

### Factors driving larval density and adult size of the threatened *Lestes macrostigma* (Odonata): keys for water management and habitat restoration

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Slightly adapted

#### *Abstract*

Freshwater insects have dramatically declined during the last decades. *Lestes macrostigma* is a damselfly of temporary brackish ponds, and is threatened in several European countries. Better understanding its larval ecological requirements is imperative to inform conservation management measures and habitat restoration programs. We studied in a set of 33 temporary ponds the effect of 14 biotic and abiotic variables (including hydroperiod, pond surface area, water salinity, oviposition plant availability and predator relative density) on *L. macrostigma* larval density and adult size at emergence. Contrarily to our expectations, salinity level and oviposition plant availability did not drive the species larval density. Instead, the later the flooding date of the pond in autumn–winter the higher the larval density in the next spring. This effect seemed mediated by the aeshnid dragonfly larvae, as the size and relative density of these predators were lower in later flooded ponds. Larval density of *L. macrostigma* also increased with decreasing pond surface area. *Lestes macrostigma* adults tended to be bigger when they emerged from ponds with higher water levels, likely because deeper waters have lower water temperatures; this larger size may positively affect adult lifespan and fecundity. Our results contribute to explaining the species strong inter-annual variation in population density and further illustrate the threat of artificial early flooding of ponds for Mediterranean species.

#### Implications for insect conservation

To improve *L. macrostigma* conservation actions, our results advocate to avoid temporary pond flooding before late autumn and to maintain high water levels until adults emerge in the next spring. Further, to increase habitat availability, our data indicate the importance of creating a network of small and deep temporary ponds.

## Introduction

Freshwater ecosystems have special conservation value given the high biodiversity they harbour compared to the total area they represent (e.g. Strayer and Dudgeon 2010; Dijkstra et al. 2014). As a possible result of recently improved water quality, abundance and biomass of freshwater insects increased in some areas (van Klink et al. 2020). Nevertheless, freshwater insects have globally declined during the last decades in Europe, especially in their biodiversity, mainly because of the intensification of agricultural practices and habitat loss (Hallmann et al. 2017; Sánchez-Bayo and Wyckhuys 2019). To take efficient conservation measures in favour of threatened species, and especially to appropriately restore their habitat, understanding their ecological requirements is imperative (Florencio et al. 2014).

In general, extinction risk is higher for specialist species, and highest for those depending on rare habitats (Gibbon et al. 2002; but see Suhonen et al. 2014). The Dark spreadwing *Lestes macrostigma* (Eversmann, 1836) (Odonata: Lestidae) is largely restricted to vernal (i.e. drying in late spring – early summer) brackish waters and has a very patchy distribution across Eurasia (Chelmick and Lambret 2020; Fig. 3.1a). It was considered in Europe as ‘Vulnerable’ but now ‘Near Threatened’ (Kalkman et al. 2010; G. de Knijf pers. comm. 2024) and even threatened in some European countries: ‘Endangered’ in France and Italy, ‘Critically Endangered’ in Bulgaria (Riservato et al. 2014; Popov 2015; UICN France et al. 2016). This species was therefore recommended to be integrated in the Habitats Directive (Kalkman et al. 2018) and is in urgent need of conservation efforts. Although its typical habitat is well known, the reasons explaining its strong inter-annual variation in population abundance and site occupancy remain unclear (e.g. Borisov 2005; Cano-Villegas and Conesa-García 2009; but see Berquier et al. 2022). In odonates, the larval stage is considered to be the critical determinant of population dynamics (Wildermuth 1994; McPeck 2008). Yet little is known about the ecological requirements of larvae of *L. macrostigma*.

Following the niche theory, ecological requirements are related to abiotic (i.e. the physio-chemical environment) and to biotic factors (Soberón and Peterson 2005). Salinity and hydroperiod (i.e. the timing and duration of the flooding season in temporary habitats) are key abiotic factors shaping freshwater insect communities (e.g. Gascón et al. 2008; Waterkeyn et al. 2008), while others such as temperature and hydrodynamics may also influence the presence and abundance of species (Hershey et al. 2009). While predation is the major biotic factor structuring freshwater animal communities (Wellborn et al. 1996), the presence of floating macrophytes can also be important, for example in determining odonate assemblages as some taxa depend on these for oviposition (Schindler et al. 2003). Hence, both abiotic and biotic factors may jointly shape freshwater insect communities. For example, Stoks and McPeck (2003a) showed that the North American *Lestes* damselflies segregate along a gradient of hydroperiod and predator presence.

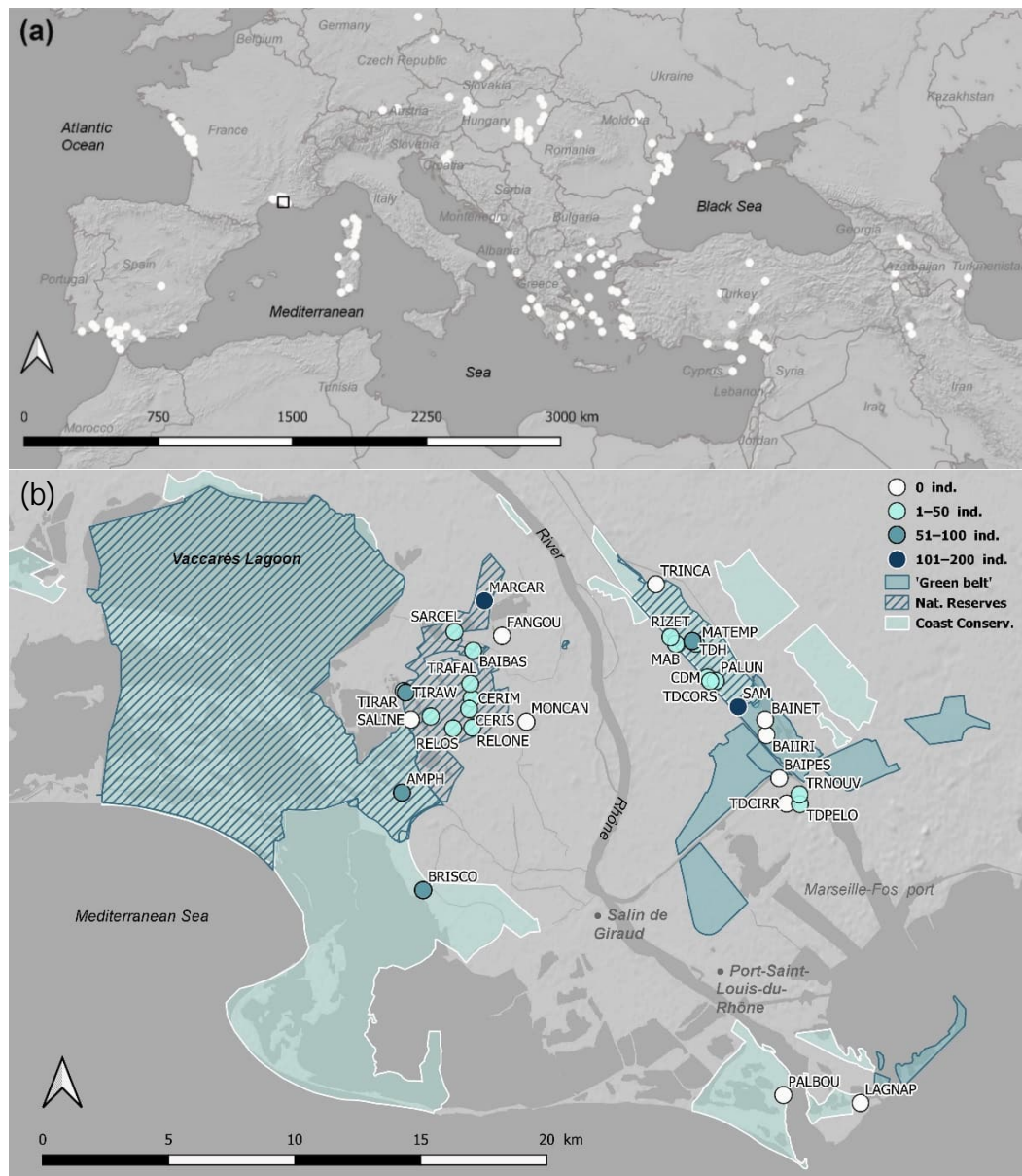


Figure 3.1 – Study area: (a) location of the Camargue (black square) with *Lestes macrostigma* occurrences (white dots) from the year 2000 onward within Europe (source: Global Biodiversity Information Facility, Occurrence download from [www.gbif.org](https://www.gbif.org), 22 April 2022, <https://doi.org/10.15468/dl.47mt7z>); (b) distribution of the 33 study ponds across the Camargue protected areas showing *L. macrostigma* larval density (i.e. total of all sweep-net and fyke counts in a given pond) recorded during the study.

The aim of this study was to improve our understanding of these requirements in order (1) to adapt the management plan of areas where the species is declining, and (2) to design effective habitat restoration projects for the recolonization of areas the species has disappeared from. Hence, we set up a field survey using temporary ponds of various salinity and hydroperiod regimes and studied the effect of several environmental variables on larval density and size at emergence. Larval abundance reflects the number of eggs laid by adults, i.e. oviposition site selection, and offspring survival (Ward

and Mill 2005). Size at emergence is a key life-history trait that is fixed in adult odonates, hence determined by larval growth conditions, and linked to adult fitness measures such as fecundity (Sokolovska et al. 2000; Stoks and Córdoba-Aguilar 2012). We selected a set of candidate environmental variables belonging to two categories. First, a set of abiotic parameters: hydroperiod and salinity that are known to affect *L. macrostigma* egg survival (Lambret et al. 2018 2021), pH which may affect odonate assemblages (Rychła et al. 2011), transparency as suspended sediments may reduce the efficiency of visual predators such as odonates (Wang et al. 2022), and dissolved oxygen (DO) which affects *Lestes* metabolism and heat tolerance (Eriksen 1984; Janssens et al. 2021). Second, a set of biotic variables concerning vegetation and animal communities: the relative density of helophyte (i.e. plants with flooded roots but aerial shoots) type of shoots preferred for oviposition (i.e. living shoots of *Bolboschoenus maritimus* (L.) Palla, dead shoots of *Juncus maritimus* Lam. and dead shoots of *J. acutus* L.; Lambret et al. 2015b, 2018), and the abundance of the submerged macrophytes (including algae) that serve as microhabitat for the larval stage (Lambret 2016a); the composition of the freshwater prey, competitor and predator communities, with a focus on odonate species as we assumed that their larvae overlap stronger in ecological niche than with other freshwater predators. We hypothesized that larval density would increase with an earlier flooding date (Lambret et al. 2018), and with intermediate salinities as these would promote higher survival (Lambret et al. 2021b) and decrease predation risk (e.g. Brucet et al. 2012). We also expected larval density to increase with a higher availability of plants preferred for oviposition, submerged macrophytes and food, and to decrease with higher densities of competitors and predators, especially Aeshnidae dragonfly larvae (Stoks and McPeck 2003a, 2003b). Finally, we expected size at emergence to decrease with increasing salinity levels (Lambret et al. 2021b) and with increasing larval density (Pierce et al. 1985).

## Methods

### *Study species*

In southern France, most of *L. macrostigma* eggs hatch between mid-March and the beginning of April (Lambret et al. 2017). Larval development is fast and lasts ca. 50–70 days (Schiel and Buchwald 2015a; Lambret et al. 2021b). Adults emerge synchronously and the emergence phase starts during the first two weeks of May and lasts ca. two weeks (Lambret 2010; Lambret and Papazian 2017). About two weeks after emergence, adults are mature and can start mating and laying eggs into plant material (endophytic oviposition) so that egg laying mainly occurs during June (Lambret 2010; Matushkina and Lambret 2011).

### Study sites and hydrology

The study took place in 2014 in the Camargue, southern France (Fig. 3.1a), where *L. macrostigma* has been yearly surveyed since 2008. We selected 33 ponds (Fig. 3.1b, Tab. S1), including ponds where the species reproduces successfully every year (i.e. permanent populations), or occasionally, or where only *L. macrostigma* adults had been observed (i.e. dispersal without reproduction). Most ponds ( $n = 27$ , 82%) were located within protected areas (i.e. the National Natural Reserve of the Camargue, the National Natural Reserve of the Marais du Vigueirat, the Regional Natural Reserve of the Tour du Valat, or other estates belonging to the French Coast Conservatory) or within the 'green belt' of the Marseille-Fos Euro-Mediterranean port, an area including natural habitats (Fig. 3.1b). We recorded the exact position of each pond using a Garmin® eTrex H GPS (Olathe, USA). We assessed the surface area of the ponds and their distance to the nearest *L. macrostigma* permanent population using the software Quantum GIS version 3.4.13 (<https://www.qgis.org>). When the pond surface area was larger than 0.1 ha ( $n = 25$  ponds, 76%), samples were made within a restricted area of 0.1 ha chosen based on the presence of oviposition plants and representative of the rather uniform shoreline of the pond.

All study ponds were temporary to vernal, i.e. with a duration of the drought period varying from few weeks to several months, respectively. The majority ( $n = 21$ , 64%) were flooded by rainfall only; eight (24%) ponds were artificially flooded by managers for conservation (seven ponds) or gaming (one pond) purposes; the water level of the four (12%) remaining ponds depended on the water level of the River Rhône. A pond was considered as flooded when its water level was at least 20 cm. We obtained the flooding date of ponds after the 2013 drought period in most cases ( $n = 24$ , 73%) from the monitoring campaign of the water levels done by wildlife managers in protected areas using limnimetric gauges. Among these ponds, 13 (54%) are flooded by rainfall only. By comparing their water levels to the data from the Météo France meteorological station of 'Arles-Valat' ( $n^{\circ}13004003$ ;  $43^{\circ}30'36''N$  |  $4^{\circ}41'38''E$ ), we were able to estimate the flooding date of the eight unmonitored ponds flooded by rainfall only. We further confirmed our estimates by an additional survey made during the 2020 autumn flooding period. We estimated the flooding date of the one unmonitored pond that depends on the level of the river Rhône using the river limnigraphic data of the 'Tarascon' station (V7200015, retrieved from the flood forecasting service website: <http://www.hydro.eaufrance.fr>) during the period 2013–2014 and established a link with the water levels we have recorded in 2014.

During the larval development period, i.e. from mid-March to mid-May, we monitored every two weeks several hydrological parameters (see Tab. S2). At the end of March 2014 (i.e. at the beginning of the egg hatching period), we measured the water level at eight randomly selected plots per pond to the nearest cm with a metallic tape measure and then calculated the mean water level. We then set a gauge in the pond to monitor water levels. We recorded the salinity 20 cm below the

water surface with a waterproof handheld meter (WTW GmbH® Cond 340i with TetraCon® 325 cell, Weilheim in Oberbayern, Germany), and dissolved oxygen (DO) and pH with another handheld meter (Eutech – Thermo Fisher Scientific® Cyberscan PD 300, Waltham, USA). We measured the water transparency using the Secchi disk method (Philippart et al. 2013).

### *Vegetation*

We measured the mean height of the submerged macrophytes in early April (i.e. at the beginning of larval development) in order to estimate the larval habitat availability. We placed a 0.5 × 0.5 m quadrat divided in four equal squares, at the eight randomly selected plots per pond. At each plot, we measured the maximum height of the submerged macrophytes in each square (hence four measures per quadrat). Based on this, we calculated the mean height of the submerged macrophyte stands per pond. Note that this method integrates the amount of vegetation cover, as zeroes (i.e. areas without vegetation) are used in calculating the mean height.

We estimated the availability of preferred oviposition plants in June (i.e. during the oviposition period). To do so, we first visually estimated the presence of helophytes by dividing the pond area into 10 squares and summing the number of squares where helophytes were present, hence resulting into a score ranging from 0.1 to 1. Second, we measured the relative frequency of preferred oviposition plants among helophytes (%) at 10 randomly selected plots within the pond (if no helophytes were present at one plot, the closest clump of helophytes was selected). At each plot, we placed the 0.5 × 0.5 m quadrat and counted the number of shoots of preferred oviposition plant shoot types (see Introduction) and other shoots. We calculated the availability of preferred oviposition plants as total cover × relative frequency × 100.

### *Animal species assemblages*

We focused on three species assemblages: (1) planktonic crustaceans, the food for *L. macrostigma* hatchlings and young larvae; (2) benthic macro-‘invertebrates’, the competitors, the prey or the predators of *L. macrostigma* larvae, with a special attention to (3) the odonate assemblage. We sampled zooplankton at the end of March 2014, i.e. at the beginning of the hatching period. At the same eight plots where the water level was measured, we took depth-integrated water samples using a tube sampler (diameter = 75 mm, height = 80 cm). We measured the height of the sampled water column to calculate the volume of each sample. Samples were sieved at 100 µm and animals were stored in 70% alcohol. In the laboratory, cladocerans, copepods and ostracods were sorted and

counted. Total planktonic crustacean densities were calculated and expressed as number of individuals per litre.

We sampled macro-‘invertebrates’, including *L. macrostigma* and other odonate larvae, and fishes (occurring in some of the ponds that are connected to the river or irrigated, and not flooded by rainfall only) between 10 and 25 April 2014 (i.e. during larval development) using two different methods, sweep-net and fykes, to maximize the estimate of taxon richness (Jurado et al. 2008). Using a sweep-net allowed us to capture plant dwelling species. At each pond, we made 10 sweeps of 5 sec, and then sorted animals in a bucket. However, strong swimmers (e.g. fishes, crayfish, aquatic Coleoptera and Heteroptera) may be underestimated with this method because they might escape during the sweeps. We therefore also used fykes (Fig. 3.2) that allow capturing species with different patterns of activity, including those active at night. We placed two fykes (0.3 cm mesh, length = 1 m, diameter = 30 cm) for 24 hr in each pond. Captured animals were stored as described above for further identification in the laboratory under a stereo microscope, except fishes and crayfish that were identified and counted in the field. Animals were identified to the family level, or to the genus or species level when possible, and counted.

We sorted and counted captured animals with respect to their expected link with *L. macrostigma* larvae in the trophic web in four categories: (0) neutral group (no relationship), (1) prey, (2) competitors and (3) predators (see *Supplementary information* for detailed method, and Tab. S3 for details about predators). While total density of predators drives encounter rates in aquatic species, predation risk is likely driven by the relative density of predators through differential predation rates (Clark et al. 2003). More generally, trophic interactions between species are determined by the likelihood of encounters (Pomeranz et al. 2019). Hence, we calculated the relative density of prey, competitors and predators relative to all captured animals, including the neutral group (see Tab. S1). For this, we used their type of diet (carnivorous and omnivorous vs phytophagous and saprophagous), and their size (estimated with a ruler as reference). Further, at the moment of animal community sampling (i.e. 10–25 April), head width of *L. macrostigma* larvae was on average  $3.04 \pm 0.57$  mm (mean  $\pm$  SD,  $n = 372$ ), corresponding to a body length ranging from ca. 10 to 25 mm. Depending on its own size, one carnivorous animal can be a prey, a competitor or a predator of *L. macrostigma* larvae. Hence, we categorized captured animals in five length size classes (0–5 mm, 6–10 mm, 11–20 mm, 21–50 mm and 50–100 mm) to help sorting them in trophic categories.

We studied in more detail the odonate assemblages. In the laboratory, captured odonate larvae were identified under a stereo microscope. Given the small size of some larvae, they could not all be identified at the species level and some were grouped (see *Supplementary information*). We then made close focus pictures of the larvae in vertical view and measured their head width using the free



software Klonk® (Image Measurement®, Cheyenne, USA, <https://www.imagemasurement.com>). Considering the mean head width of *L. macrostigma* larvae (see above), larvae of other odonate species were considered as prey or predators when their head width was below 2 mm or above 4.5 mm, respectively, and as competitors when in-between.



Figure 3.2 – Macro-‘invertebrates’ were sampled using two fykes set at each pond (a) in addition to net sweeps; captured animals were collected after 24 hrs (b) (© P. Lambret).

#### *Size of Lestes macrostigma at emergence*

Adults of the species emerged in large numbers at 15 of the 33 studied ponds. Using their typical coloration, we were able to estimate the age of immature adults and captured those that emerged few hours before (Lambret 2013). We recorded their sex and, using dial calipers (Ecotone® Measy 150, Sopot, Poland), we measured the length of their hind right wing (from the basis to the apex) to the nearest 0.1 mm, as a proxy of body size in odonates (Sacchi and Hardersen 2013).

#### *Statistical analyses*

All analyses were performed in R 3.6.3 (R Core Team 2020). Figures were made using Quantum GIS and the R package ‘ggplot2’ (Wickham et al. 2020). As pond pH and DO values vary during the day (e.g. Reddy 1981), we corrected them for each pond as if they were measured at the same time of the day (see *Supplementary information*). We used a Mantel test to assess whether *L. macrostigma* larval densities were spatially auto-correlated (Legendre 1993). Using the ‘Hmisc’ package (Harrell 2020), we quantified the Pearson’s *r* correlation coefficients between the measured environmental variables (Tab. 3.1). A threshold value of  $r = 0.7$  was used to assess whether two variables were correlated

enough to be considered as redundant. Based on this criterion, none of the explanatory variables was removed from the subsequent analyses.

To disentangle which are the main environmental drivers in shaping *L. macrostigma* larval density and assess their relative importance, we used a model averaging approach (Grueber et al. 2011; Burnham and Anderson 2002) on a fully parametrized multiple generalized linear regression model with as explanatory variables: (1) pond surface area ( $\log_e$ -transformed), (2) distance to the nearest permanent population ( $\log_e$ -transformed), (3) flooding date (expressed as week number, with the weeks of the 1<sup>st</sup> January 2013 and 2014 being respectively 1 and 53), (4) mean water level at the moment of *L. macrostigma* larvae sampling (i.e. 17–24 April), (5) salinity, (6) corrected pH, (7) corrected DO, (8) transparency, (9) submerged macrophyte height, (10) relative abundance of preferred oviposition plants, (11) planktonic crustacean total densities (plankton relative densities could not be calculated because plankton and macro-'invertebrates' were not sampled at the same moment), and the relative densities of (12) prey, (13) competitors and (14) predators (see Tab. S1 for units). All explanatory variables were scaled (standard deviation = 1 and mean = 0) to allow comparison of estimated regression coefficients (i.e. effect sizes). Abundances of *L. macrostigma* were modelled with a negative-binomial distribution and loglink function using the 'glmmTMB' package (Magnusson et al. 2020). Based on this fully parametrized model, a model averaging was performed using the package 'MuMIn' (Bartoń 2020). Model selection was performed by comparing all competing regression models, including all possible combinations of explanatory variables. The selection of the top models was based on the lowest AICc values. Only those models within two AICc units ( $\Delta < 2$ ) of the most parsimonious model were retained (Grueber et al. 2011). Averaged regression coefficients estimated by the model averaging approach are given as estimates with their 95% confidence intervals, together with their statistic and p-values. Effects were considered significant when p-values were below Fisher's 0.05 threshold. Yet, we also gave full attention to  $p\text{-value} \leq 0.1$  as p-values should be regarded as graded measures of the strength of the statistical signal (Amrhein et al. 2017). The relative importance of each explanatory variable was quantified by summing the Akaike weights across all top models containing the corresponding explanatory variables.

The distribution of larval size of the different odonate taxa was assessed using a violin chart. We then tested for the effect of flooding date on the mean size of Aeshnidae larvae using a linear model in which we included site as fixed factor, and not as random effect, because of the small number of Aeshnidae we found at some sites (see Tab. S4). We further tested for the effect of relative densities of larger and smaller Aeshnidae (i.e. above or below a threshold of 4.5 mm head width) on the density of *L. macrostigma* using a linear model with a negative-binomial distribution and the loglink function using the 'glmmTMB' package (Magnusson et al. 2020).

We tested using a two-way ANOVA whether *L. macrostigma* wing lengths (i.e. size at emergence) varied across ponds and between females and males because sexual size dimorphism is common in odonates (Corbet 2004). Then, to tease apart which environmental variables played a role in explaining adult size at emergence, a simple linear mixed model was performed, with as explanatory variables those environmental variables which larvae were directly exposed to: water level, salinity, corrected pH, corrected DO, transparency, submerged macrophyte height, planktonic crustacean densities, and relative densities of prey, competitors and predators. We also included as explanatory variables flooding date because in insects, desiccation may select for longer development times, possibly resulting in an increase of mean adult size (Gefen et al. 2006), and *L. macrostigma* larval density as we expected intraspecific competition to have an effect on adult size (Pierce et al. 1985). We used a nested design including 'pond' as a random effect to take into account that several measured individuals emerged from the same pond, and included 'sex' as fixed factor to take into account the result of the two-way ANOVA.

## Results

There was no spatial autocorrelation of larval densities in our dataset ( $p = 0.378$ ; Fig. 3.1b). Pond surface area varied from 191 m<sup>2</sup> to 61.6 ha (see Tab. S1). Flooding date varied from early August (3 ponds; depending on river level) or mid-September (1 pond managed for waterfowl gaming) to mid-December or mid-January (12 ponds; depending on rainfall only). Salinity ranged from 0.3 to 13.4 g/L during the hatching period (measured on 18–24 March) and from 1.1 to 21.7 g/L during the main emergence period (measured on 6–13 May) (see Tab. S2). *Lestes macrostigma* larvae were not detected in 10 of the 33 ponds. Among the 23 ponds where *L. macrostigma* was present, larval density varied from 1 to 196 individuals (totals of all sweep-net and fyke counts per pond).

The model averaging approach selected eight top models retaining seven variables in the averaged model to explain larval densities: pond surface area ( $\log_e$ -transformed), distance to the nearest permanent population ( $\log_e$ -transformed), flooding date, mean water level, corrected DO, and relative densities of prey and competitors (Tab. 3.2). The variation in the *L. macrostigma* density among ponds was significantly explained by two variables: larval density decreased with increasing pond surface area ( $-0.821$ , 95% CI:  $[-1.525; -0.117]$ ,  $z = 2.285$ ,  $p < 0.05$ ; Fig. 3.3a) and increased with later flooding dates ( $1.174$ , 95% CI:  $[0.382; 1.966]$ ,  $z = 2.905$ ,  $p < 0.01$ ; Fig. 3.3b). Both variables

Table 3.1 – Pearson correlation matrix between measured explanatory variables: r-values are given on top-right and p-values on bottom left halves of the table. (Area: pond surface area; Distance: to the nearest *L. macrostigma* permanent population; Water level: mean pond water level during the animal communities sampling session; Salinity: level during *L. macrostigma* hatching period; Macrophyt.: macrophyte mean height; Ovip. plants: weighted cover of preferred oviposition plants; Plankton: planktonic crustacean density; Prey, competitors and predators: relative density to all captured animals).

	Geography		Hydrology			Vegetation					Animal communities			
	Area	Dist.	Flood.	Water level	Sal.	pH	DO	Visib.	Macroph.	Ovip. plants	Plankton	Prey	Comp.	Pred.
Area		0.22	−0.12	−0.47	−0.16	0.18	0.22	−0.25	−0.09	0.20	0.09	−0.13	0.06	−0.06
Distance	0.218		−0.07	−0.16	0.12	−0.40	−0.40	0.22	−0.16	0.33	−0.06	−0.26	−0.30	0.19
Flooding date	0.496	0.709		0.16	0.00	0.09	0.22	0.26	0.20	−0.09	−0.25	0.34	−0.22	0.44
Water level	0.006	0.383	0.364		0.22	−0.34	−0.06	0.32	0.06	−0.33	−0.22	−0.04	−0.07	−0.16
Salinity	0.361	0.517	0.997	0.225		−0.12	−0.19	−0.27	−0.42	−0.31	−0.21	−0.27	−0.28	−0.30
pH	0.315	0.022	0.622	0.053	0.509		0.57	−0.22	−0.07	−0.26	−0.30	0.39	−0.16	0.18
Dissolved O <sub>2</sub>	0.217	0.022	0.222	0.737	0.290	0.001		−0.03	0.12	−0.26	−0.22	0.40	−0.14	0.09
Transparency	0.165	0.220	0.147	0.072	0.125	0.223	0.885		0.24	0.02	−0.05	0.07	−0.13	0.36
Macroph.	0.630	0.367	0.258	0.733	0.014	0.687	0.495	0.177		0.27	0.26	0.10	0.24	0.24
Ovip. plants	0.273	0.060	0.618	0.057	0.080	0.144	0.146	0.926	0.124		0.32	−0.32	0.15	0.41
Plankton	0.615	0.739	0.159	0.210	0.249	0.088	0.227	0.769	0.146	0.066		−0.31	0.48	−0.09
Prey	0.478	0.150	0.055	0.832	0.132	0.023	0.020	0.679	0.580	0.071	0.084		−0.46	0.15
Competitors	0.741	0.086	0.220	0.712	0.120	0.387	0.431	0.483	0.170	0.402	0.005	0.007		−0.18
Predators	0.760	0.280	0.011	0.371	0.085	0.329	0.622	0.041	0.183	0.019	0.629	0.405	0.318	

had a similar effect size: estimated regression coefficient for flooding date = 1.174 vs estimated regression coefficient for area =  $-0.821$ , with  $|1 - |1.174|| \approx |1 - |-0.821||$  (Tab. 3.2). In terms of relative importance, the flooding date and the surface of the pond ( $\log_e$ -transformed) were also the most important explanatory variables, as they were included in all selected models within two AICc units (Tab. 3.2).

Only Aeshnidae showed a clear bimodal distribution (Fig. 3.4), suggesting the occurrence of two cohorts. These with head larger than 4.5 mm all belonged to the species *Anax parthenope* (Selys, 1839). The mean size of Aeshnidae varied significantly across sites (in 21 ponds out of 33:  $|t| \in [2.052; 5.805]$ ;  $p \in ]0; 0.043]$ ) and with the flooding date ( $t = -3.268$ ,  $p = 0.002$ ): the sooner the flooding of the pond in autumn–winter the bigger some of the Aeshnidae larvae, i.e. the *A. parthenope* (see above). Further, the relative density of larger Aeshnidae had a significant negative effect on *L. macrostigma* larval density while this negative effect was not significant with the relative density of smaller Aeshnidae (larger Aeshnidae:  $z = -104.939$ ,  $df = 1$ ,  $p = 0.035$ ; smaller Aeshnidae:  $z = -26.601$ ,  $df = 1$ ,  $p = 0.501$ ).

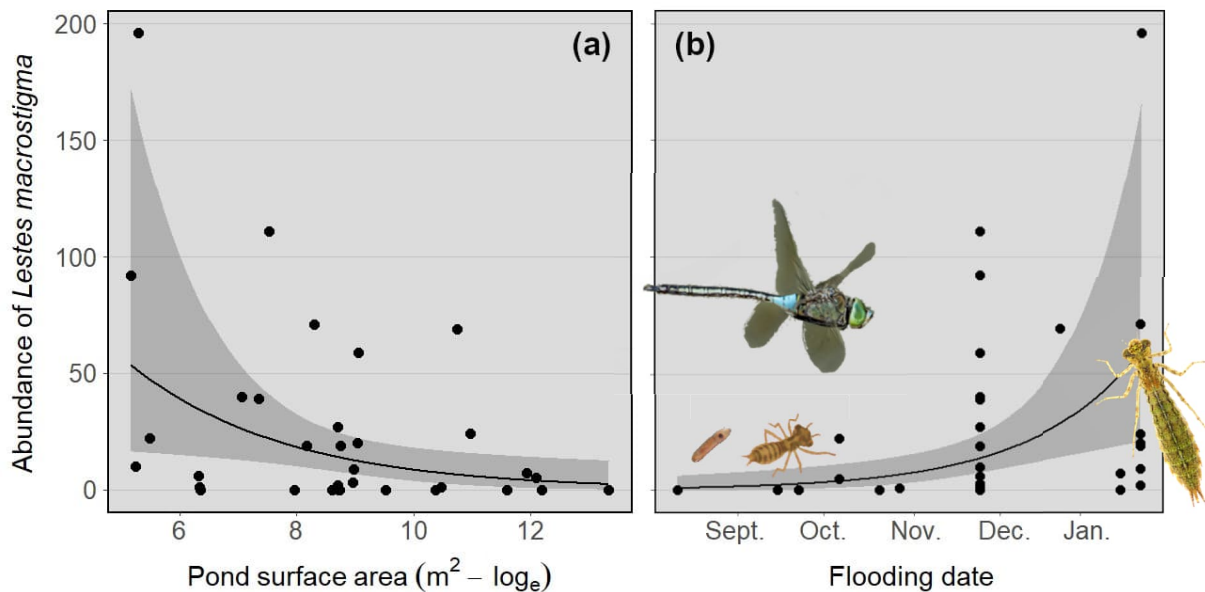


Figure 3.3 – Effect of pond surface area (a) and of pond flooding date in autumn–winter (b) on *Lestes macrostigma* larval density in the next spring. The black curve indicates the fitted density and the dark grey band the 95% confidence intervals estimated by the averaged model. The black dots indicate the observed values. Larval density is the total of all sweep-net and fyke counts in a given pond. The dragonfly *Anax parthenope* indicates the period (i.e. Sept.-Nov.) during which this species may lay eggs in a pond, at least if the pond is already flooded. When these eggs are laid earlier, as they hatch rapidly, larger aeshnid larvae may be present in the pond the next year, during *L. macrostigma* larval development.

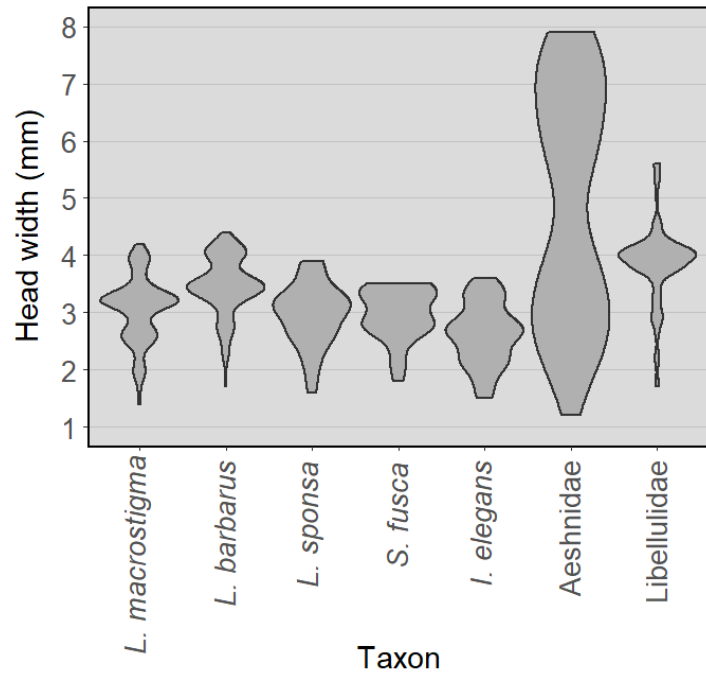


Figure 3.4 – Size distribution of individuals of the different odonate taxa sampled during 17–24 April across the 33 study ponds. Note that Aeshnidae show two cohorts, with the bigger being composed of *Anax parthenope* only.

A total of 909 recently emerged adults were sampled. Females were significantly bigger than males (two-way ANOVA:  $F = 386.55$ ,  $df_1 = 1$ ,  $df_2 = 908$ ,  $p < 0.001$ ): on average hind wing length was 25.47 mm ( $SD = 0.94$ ,  $n = 447$ ) in females and 24.59 mm ( $SD = 0.98$ ,  $n = 462$ ) in males. Size at emergence differed significantly across ponds (two-way ANOVA:  $F = 61.72$ ,  $df_1 = 15$ ,  $df_2 = 894$ ,  $p < 0.001$ ). However, we could not clearly identify which environmental variables were driving these differences across ponds (Tab. 3.3). The only pattern was that individuals tended to be bigger when they emerged from ponds with higher water levels (Tab. 3.3:  $t = 3.477$ ,  $df = 2$ ,  $p = 0.074$ ).

### Discussion

*Lestes macrostigma* is well known for its strong inter-annual variation in population abundance and site occupancy (e.g. Borisov 2005; Cano-Villegas and Conesa-García 2009). Berquier et al. (2022) showed that the duration of the spring flooding period drives, at least partially, the number of adults that emerge from the aquatic larval environment. We here present important findings concerning the density of larvae, the critical life stage determining odonate population dynamics (Wildermuth 1994; McPeck 2008). We found two environmental variables having a significant effect on *L. macrostigma* larval density with similar effect sizes: the surface area of the pond and the flooding date of the pond in autumn–winter.

Table 3.2 – Model averaging summary results showing the selected environmental variables (with their relative importance) explaining variation in the density of larvae of *Lestes macrostigma*. Relative importance of each explanatory variable was quantified by summing the Akaike weights across all top selected models. Estimated coefficients were weighted and standardized, allowing the comparison of the size and direction (positive or negative) of their effect on *L. macrostigma* density. Variables having a significant effect are presented in bold.

Parameter	Coefficient	Adjusted SE	z	p	Relative importance
Flooding date	1.174	0.404	2.905	0.004	1
Pond surface area	-0.821	0.359	2.285	0.022	1
Dissolved O <sub>2</sub>	0.238	0.400	0.595	0.552	0.36
Relative density of prey	-0.160	0.318	0.503	0.615	0.31
Water level	-0.114	0.270	0.423	0.672	0.23
Relative density of competitors	0.047	0.178	0.267	0.790	0.12
Distance to the nearest population	-0.024	0.118	0.203	0.839	0.09

Table 3.3 – Summary results of the linear mixed model testing for effects of sex and selected environmental variables on *Lestes macrostigma* size at emergence.

Variable	Estimated effect	SE	df	t	p
Sex	-0.901	0.046	893	-19.664	< 0.001
Water level	1.939	0.558	2	3.477	0.074
Flooding date	-0.270	0.250	2	-1.079	0.393
Salinity	-1.134	0.499	2	-2.273	0.151
pH	-0.356	0.332	2	-1.074	0.395
Dissolved O <sub>2</sub>	0.433	0.263	2	1.645	0.242
Transparency	0.276	0.227	2	1.216	0.348
Hydrophyte height	-1.200	0.436	2	-2.752	0.111
Plankton density	0.211	0.218	2	0.967	0.436
Relative density of prey	0.027	0.177	2	0.150	0.894
Relative density of competitors	0.615	0.249	2	2.471	0.132
Relative density of predators	1.325	0.560	2	2.365	0.142
<i>L. macrostigma</i> density	0.083	0.198	2	0.420	0.715

Pond surface area had a negative effect, with smaller ponds showing higher larval density. Larval abundance and density should be driven, amongst other factors, by the number of females laying eggs the previous year, which depends on the oviposition site selection by the adult. Although oviposition plants play a major role during oviposition site selection (Lambret et al. 2015a, 2015b, 2018), we did not find the preferred oviposition plant availability to have a significant effect on larval density. This suggests that, even with low availability of preferred oviposition plants, smaller ponds may be especially selected by adults, with the few preferred oviposition plants receiving high number of laid eggs and therefore leading to a higher larval density in the next spring. Further, adults could select smaller ponds during oviposition because pond size may be used as a proxy for presence of predators such as fish or water beetles (e.g. Wildermuth 1994; Pearman 1995). Yet, despite higher *L. macrostigma* larval densities in smaller ponds, larger ponds may overall harbour larger populations (see Lambret et al. 2023a, 2023b; Fig. I.3).

Pond flooding date had a positive effect on larval density: the later the date of flooding of the temporary habitat after the summer–autumn drought season, when *L. macrostigma* is at the egg stage, the higher the larval density the following spring. Lambret et al. (2018) showed that its life cycle allows the species to cope with drought season lasting up to the winter, but that egg survival decreases with later flooding dates. Hence, this current finding is contrary to our expectation based on egg survival, and suggests that other mechanisms, related to the indirect positive effect of a later flooding date, drive larval survival. Hydroperiod is known as a strong driver of freshwater insect communities (e.g. Timms 2012; Whiles and Goldowitz 2005). When focusing on trophic links, the relative density of prey was moderately and positively correlated to pond flooding date ( $r = 0.34$ , Tab. 3.1), suggesting a higher availability of prey in ponds that flood later. Integrating relative densities of prey and competitors (which both drive *L. macrostigma* food availability) as variables improved the average model explaining *L. macrostigma* larval density but these variables were not significant (Tab. 3.2). Instead, the relative density of larger Aeshnidae had a significant negative effect on *L. macrostigma* larval density. We also found that the later the flooding date the smaller the aeshnid larvae are in the next spring (see *Supplementary information*). While eggs of *A. affinis* and *A. mixta* hatch after winter diapause, the eggs of *A. parthenope* hatch a couple of weeks after oviposition (Schiel and Buchwald 2016b), which occurs until November in the Camargue (Blanchon 2017; Fig. 3.3b) and odonate larvae start their growth immediately after hatching (Corbet 2004). Hence, the sooner the eggs of *A. parthenope* are laid the bigger its larvae are in the next spring (Fig. 3.4), thereby increasing their predation ability on *L. macrostigma* larvae. This suggests that the higher density of *L. macrostigma* in ponds with a late flooding date is mediated by their lower density in larger Aeshnidae. This is supported by Lambret et al. (2021) who showed that the species fundamental niche, which includes fresh waters, is shifted



toward ponds of higher salinity levels (realized niche), likely because of higher density of predators in fresh waters (e.g. Brucet et al. 2012). This scenario resembles that of the vernal pond-specialist *L. dryas*: Stoks and McPeck (2003a) showed this sister species is fully absent from temporary ponds (these having a longer hydroperiod than vernal ponds) and permanent water bodies because of predation risk, especially by Aeshnidae which are the top invertebrate predators in these habitats. This was driven by the vernal pond *L. dryas* not reacting to aeshnid predation risk and fully capitalising on active foraging to achieve a fast development and growth to complete its life cycle before pond drying (Stoks and McPeck 2003b). The vernal pond *L. macrostigma* even has faster growth and development than *L. dryas* (Schiel and Buchwald 2015a, 2016a), suggesting it may follow the same risky active foraging strategy and is therefore sensitive to predation by bigger Aeshnidae.

When considering the environmental variables potentially driving *L. macrostigma* larval density, we expected that density would increase with increasing salinities because (1) larval survival in the laboratory increased with salinity from 0.5 to 8 g/L (Lambret et al. 2021b) and (2) relative density of predators would be lower at higher salinity. However, we found salinity to have no significant effect on larval density. One reason this prediction did not hold was that we found no support for the second assumption. Indeed, and in contrast to the literature (e.g. Brucet et al. 2012), predator relative density was only moderately negatively correlated to salinity ( $r = -0.30$ , Tab. 3.1). A second reason may be that our study lacked ponds with very high salinity levels. In the present study, the salinity levels were below 8 g/L in 30 of the 33 studied ponds during the hatching period and were above 10 g/L in six ponds only during the emergence period (see Tab. S2). However, the species can occur in ponds with higher salinity levels: Lambret et al. (2009) observed adults emerging from ponds having ca. 21–23 g/L salinity, and in 2022 the species even emerged from water ca. 28 g/L salinity yet with low success (Lambret pers. obs.). This shows that at least some larvae are able to cope with high salinities during a bout of their development, despite negative effects on survival (Lambret et al. 2021b). Including such higher salinity ponds where the species occurs might have revealed a positive effect of higher salinity levels on larval density.

Another expected effect of increasing salinity was its negative effects on adult traits such as size at emergence (Lambret et al. 2021b). However, we found adults emerging from some high salinity ponds at a bigger size than those emerging from some low salinity ponds. This suggests that at least another environmental variable, we could not clearly identify here, may have overruled the negative effect of salinity on size at emergence. Our results suggest that adults could emerge at a bigger size when growing in ponds with higher water levels. Conversely, adults could emerge from shallower waters at a smaller size, i.e. earlier than when fully grown, to avoid drought while still at the larval stage. However, accelerated development as a direct adaptive response to the risk of habitat drying in

temporary pond (i.e. decreasing water levels) has never been shown in *Lestes* damselflies (De Block & Stoks 2005b). Instead of such a direct effect, shallow waters are likely to drive size at emergence indirectly through water temperature, an environmental variable we did not measure in our study. Indeed, temperature is typically higher in shallow waters receiving a similar solar radiation than deeper waters. Higher temperatures are well known to accelerate the larval pace-of-life of odonates and other insects, with increased growth rate and decreased development time, leading to emergence at a smaller size or lower body mass (Verberk et al. 2021; Tüzün and Stoks 2022; for another *Lestes*: De Block & Stoks 2003) with cascading negative effects on adult longevity and reproductive success (Sokolovska et al. 2000). For example, on 12 May 2022, most of *L. macrostigma* adults had already emerged from a pond with a water level of ca. 10 cm, while no adults were visible at another pond with ca. 35 cm of water depth, the latter yet containing hundreds of last instar larvae of *L. macrostigma* (Lambret pers. obs.). This water level-mediated effect of temperature was most probably obscured in our study by other interacting variables that affect size at emergence, such as salinity level, intra-guild competition, availability of prey or density of predators (Schaffner & Anholt 1998; Sniegula et al. 2019; Lambret et al. 2021b).

By identifying the role of the timing of pond flooding for larval density and taking into account the high inter-annual variability of autumn rainfall, the current study lays an important basis for understanding the considerable, and so far somewhat enigmatic, year-to-year variation in density of *L. macrostigma*. The here identified two key factors affecting larval density, flooding date and pond surface area, will be crucial to build population dynamic models of the species. Hereby, meteorological data over several years could allow checking for the effect of flooding date at a larger geographic scale. Specifically, our results contribute to the assessment of the consequences of artificial water regimes in the Mediterranean. Since the containment of the river Rhône and the Mediterranean Sea in the middle of the 19<sup>th</sup> century, the hydro-sedimentological dynamics of the delta have been fixed, with no possibility of natural flooding by river and marine intrusion (Provansal et al. 2003). Besides rainfall, the flooding of wetlands in the Camargue is controlled and depends on land use (agriculture, waterfowl gaming, wildlife conservation). Noteworthy is the water regime of the marshes which are managed for hunting purposes: large amounts of fresh water are artificially injected from late July or early August (a drought period under the Mediterranean climate) in order to trigger the growth of aquatic plants and therefore attract the waterfowl at the opening of the hunting season in late August. Tamisier and Grillas (1994) showed that such water regime leads to a loss of plant diversity and to a shift from diverse Mediterranean to simplified continental plant communities. Artificial fresh water inputs in Mediterranean wetlands also strongly affect the benthic assemblages (Gascón et al. 2005). We here provide an example of the negative effect of such artificial early flooding for a threatened insect that

should be integrated into the EU Habitats Directive (Kalkman et al. 2010, 2018). During the last centuries, the development of urbanisation along the Mediterranean coast and of agriculture and industry has dramatically decreased the suitable habitat for *L. macrostigma*, so that the core populations are entirely restrained to protected and managed areas. Water management appears especially important because of climate change inducing a decrease in rainfall and an increase in its unpredictability (Polade et al. 2017; Zittis 2018).

In addition to Berquier et al. (2022), our results provide guidelines about water management to conserve the species. First, to maximize larval survival, management plans in the Camargue region should target to flood marshes and ponds from late autumn (November) to late spring (June). This timing and duration should be adjusted to the species' phenology in other regions where the flying period may start earlier (e.g. Díaz-Paniagua et al. 2015) or end later (e.g. Benken and Raab 2008). Further, wildlife managers are encouraged to maintain water levels of at least 30 cm during spring in order to maximize adult size at emergence, and therefore reproductive success. Second, in addition to the regional preservation of larger and shallow water bodies, recreating a network of ponds by ecological engineering should be engaged in order to provide stepping-stones across the fragmented distributional range of *L. macrostigma* (Shuey 2013). These ponds should be smaller (i.e. ca. 200–1000 m<sup>2</sup>) to maximize oviposition site selection by adults.

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### *Author's contributions*

Conceptualization: PL, RS; Funding acquisition: PL; Project administration: PL; Methodology: PL, MJ, RS; Validation: PL, RS; Investigation: PL; Data curation: PL, MJ; Formal analysis: PL, MJ; Visualization: PL, MJ; Writing | original draft: PL; Writing | review & editing: PL, MJ, RS; Resources: PL, MJ, RS; Supervision: RS.

### Supplementary information

#### Correction of pH and Dissolved Oxygen measured values

We measured DO across ponds at different day times. Values of pH and DO vary during the day especially because of the photosynthesis activity of submerged macrophytes when solar radiation crosses the water surface, i.e. with no floating vegetation (Romaine and Boyd 1979; Reddy 1981). Hence, we corrected for each pond the pH and DO values as if they were measured at the same time of the day (i.e. 4:00 PM) using a Generalised Linear Model (GLM).

#### Animals sorting regarding their trophic link with *Lestes macrostigma* larvae

Among phytophagous species, snails (Mollusca: Gastropoda, 33% of all phytophagous individuals), amphipods (Gammaridae, 26%) 6–20 mm and adults of water scavenger beetles (Hydrophilidae, 4%) 6–20 mm which are likely hard to capture by *L. macrostigma* larvae were considered as neutralist. Other phytophagous species up to 20 mm, mainly midges larvae (Chironomidae, 20%) and mayfly larvae (Baetidae, 9%), were considered as prey. All phytophagous animals larger than 20 mm were considered as neutralist.

Carnivorous and omnivorous animals smaller than 5 mm were considered as prey, those between 6 and 10 mm as competitors, and those larger than 10 mm as predators. Within the group of predators, we made further specifications for crayfish and fish. The only crayfish species in the ponds, *Procambarus clarkii* (Girard, 1852), is an invasive species well-known to have in the adult stage a negative impact on predator macro-invertebrate communities, especially on odonates (Gutiérrez-Yurrita et al. 1998; Miyake and Miyashita 2011; Rodríguez-Pérez et al. 2016). We therefore only considered crayfish >50 mm as predators. All size classes of following four fish species were considered as predators: Crucian carp *Carassius carassius* (Linnaeus, 1758) (Penttinen and Holopainen 1992), Largemouth black bass *Micropterus salmoides* Lacépède, 1802 (Godinho and Ferreira 1994), European eel *Anguilla anguilla* (Linnaeus, 1758) (Ezzat and El-Seraffy 1977) and Black bullhead *Ameiurus melas* (Rafinesque, 1820) (Rosecchi et al. 1997). Three other fish species were considered as competitors as these have not or only rarely been recorded to feed on damselfly larvae: the Mosquitofish *Gambusia affinis* (Baird and Girard, 1853) (Crivelli and Boy 1987); the Topmouth gudgeon *Pseudorasbora parva* (Temminck and Schlegel, 1846) (Wolfram-Wais et al. 1999; Xie et al. 2001), and the Three-spined stickleback *Gasterosteus aculeatus* Linnaeus, 1758 (Hynes 1950).

In the laboratory, caught odonate larvae were identified under a stereo microscope. Given the small size of some larvae, they could not all be identified at the species level. Hence, we grouped (1) *Aeshna affinis* (Vander Linden, 1820), *Anax parthenope* (Selys, 1839) and unidentified Aeshnids – possibly including *A. mixta* Latreille, 1805 – together as Aeshnidae, and (2) *Crocothemis erythraea* (Brullé, 1832), *Sympetrum fonscolombii* (Selys, 1840), *S. striolatum* (Charpentier, 1840) and unidentified libellulids as Libellulidae. *Orthetrum* sp (n = 3) were not considered in this analysis because these bottom dwellers (Suhling et al. 2005) are unlikely to encounter the plant dwelling *L. macrostigma* (Lambret 2016a).

Table S1. Characteristics of sampling ponds including the environmental variables used for the fully parametrized model and following model averaging approach. Lat. | Long.: latitude | longitude (° WGS84); Area: pond surface area (m<sup>2</sup>); Dist.: distance to the nearest *L. macrostigma* permanent population (km); Flood: flooding date (expressed as week number, with the weeks of the 1<sup>st</sup> January 2013 and 2014 being respectively 1 and 53); Water level: mean pond water level (cm) during the animal communities sampling session (i.e. 17–24 April); Sal.: salinity level during hatching period (g/L); pH: pH at 4:00 PM; DO: dissolved O<sub>2</sub> at 4:00 PM; Transp.: transparency (cm); Macroph.: macrophyte mean height (cm); Ovip. plants: weighted cover of preferred oviposition plants (%); Plankton: planktonic crustacean density (individuals/L); Neutr., Prey, Comp., and Pred.: neutralists, prey, competitors and predators density (total number of individuals of all sweep-net and fyke counts in a given pond); *L. mac.*: *L. macrostigma* larval density (individuals). Ponds are sorted regarding decreasing density of *L. macrostigma*. The range of each variable (min and max values) is given in bold characters. (To be continued.)

Pond	Geography			Hydrology						Vegetation			Animal communities					
	Lat.	Long.	Area	Dist.	Flood.	Water level	Sal.	pH	DO	Transp.	Macroph.	Ovip. plants	Plankton	Neutr.	Prey	Comp.	Pred.	<i>L. mac.</i>
MARCAR	43.52841	4.68186	200	5.0	55	28.6	3.3	8.2	12.5	30.7	20.1	47.7	69.8	3	370	37	43	196
SAM	43.49087	4.80594	1857	0.0	47	22.7	2.4	8.3	14.3	23.8	13.1	28.2	165.3	260	202	477	7	111
TDH	43.51413	4.78356	176	0.0	47	51.9	7.9	8.9	16.3	28.6	21.3	0.5	70.3	0	122	233	10	92
TIRAW	43.49592	4.64343	4053	3.0	55	22.8	4.0	8.6	13.9	25.3	10.1	65.6	53.4	10	163	86	36	71
AMPH10	43.46043	4.64158	46440	3.9	51	8.8	2.8	9.1	13.8	31.7	5.7	2.9	59.9	43	140	112	22	69
BRISCO	43.42588	4.65193	8645	6.8	47	8.0	6.2	8.8	12.6	21.0	7.2	90	138.0	857	764	658	604	59
TIRAR	43.49649	4.64238	1164	3.1	47	52.5	9.6	8.8	11.3	33.5	0.0	2.6	42.8	1471	7	14	11	40
RIZET	43.51557	4.77283	1558	0.0	47	25.9	2.9	9.7	15.1	31.8	20.6	20	80.5	35	153	164	34	39
RELONE	43.48349	4.67592	6030	0.0	47	25.2	4.3	8.9	13.2	23.5	10.5	80	145.0	4	147	468	28	27
SARCEL	43.51742	4.66722	57936	3.8	55	16.7	3.6	7.9	10.2	37.5	21.0	77	317.5	39	92	162	28	24
MAB	43.51312	4.77552	243	0.2	40	29.4	4.1	9.7	15.5	23.8	3.5	0	65.9	0	584	27	14	22
CERIM	43.49382	4.67561	8421	1.2	55	15.9	2.0	9.7	16.6	24.0	14.8	5.2	21.4	12	288	81	71	20
TAMARG	43.48744	4.65558	6352	1.8	55	27.9	2.6	9.5	12.5	24.7	20.7	57.6	77.0	11	155	48	51	19
TRAFAL	43.49904	4.67494	3539	1.7	47	1.4	4.8	10.0	13.6	20.5	3.3	11.4	105.8	0	553	33	4	19
TRNOUV	43.45978	4.83586	191	4.2	47	28.9	6.0	8.4	10.9	29.3	11.3	47.9	78.9	10	120	65	28	10
CERIS	43.49009	4.67435	7873	0.8	55	16.1	3.2	9.9	14.9	31.3	13.4	12.7	94.9	3	132	25	42	9
RELOS	43.48324	4.66653	152495	0.4	54	21.5	4.4	9.9	17.8	20.3	7.1	10	139.8	2	185	40	6	7
TDCORS	43.49996	4.79223	558	1.5	47	21.6	2.4	8.4	11.0	27.5	5.6	3	129.7	33	808	782	30	6
CDM	43.50123	4.79081	179768	1.5	40	28.8	2.6	9.2	14.8	20.0	14.7	25.4	145.6	53	116	214	6	5

Table S1. (Continued.)

Pond	Geography			Hydrology				Vegetation					Animal communities					
	Lat.	Long.	Area	Dist.	Flood.	Water level	Sal.	pH	DO	Transp.	Macroph.	Ovip. plants	Plankton	Neutr.	Prey	Comp.	Pred.	<i>L. mac.</i>
MATEMP	43.51324	4.78521	7818	0.2	47	19.5	8.0	9.1	13.5	10.5	7.5	18.3	38.1	23	1204	186	26	3
BAIBAS	43.51081	4.67633	6031	3.0	55	30.3	4.1	8.8	15.9	30.3	31.8	0	108.1	28	574	82	16	2
PALUN	43.50002	4.79495	35135	1.4	43	26.4	2.3	8.6	12.4	18.8	8.8	25.2	98.6	30	383	37	5	1
TDPELO	43.45639	4.83611	565	4.6	47	37.2	3.2	8.3	9.8	35.5	12.2	26.8	19.7	14	45	62	6	1
BAIIRI	43.48082	4.81971	13720	1.6	32	11.9	3.0	9.3	9.5	16.0	12.6	20.1	148.8	65	540	2253	10	0
BAINET	43.48625	4.81911	6094	1.2	32	15.3	3.2	8.1	12.9	20.7	15.5	39.9	408.8	251	229	1487	29	0
BAIPES	43.46552	4.82598	5457	3.3	47	27.4	11.3	8.5	13.2	16.3	0.0	8.9	16.7	1978	90	74	10	0
FANGOU	43.51602	4.69043	198826	3.8	38	8.9	3.1	8.8	13.5	30.3	14.9	100	51.8	369	165	47	49	0
LAGNAP	43.35029	4.86571	615912	16.3	37	8.1	8.0	8.9	14.4	25.3	2.5	17.1	3.5	237	165	24	7	0
MONCAN	43.48554	4.70239	2895	2.0	47	62.0	0.8	8.1	13.8	40.5	10.0	0	45.3	31	757	12	85	0
PALBOU	43.35313	4.82801	6279	15.4	42	21.1	0.3	8.6	13.0	29.7	10.0	100	202.6	523	105	213	108	0
SALINE	43.48630	4.64597	108186	2.6	54	33.1	6.1	8.7	13.8	19.0	0.0	0.6	73.5	3	17	47	2	0
TDCIRR	43.45655	4.82952	579	4.3	47	41.1	14.1	8.1	8.9	19.3	6.4	3.5	151.1	1524	271	2	4	0
TRINCA	43.53425	4.76569	31957	2.3	47	26.1	1.4	8.6	12.1	12.7	21.9	80	108.4	109	67	166	7	0

Table S2. Hydrological parameters monitored during *Lestes macrostigma* larval development. Session 1: 18–24 March 2014, session 2–4 April, session 3: 17–24 April, session 4: 6–13 May, session 5: 28 May–2 June 2014.

Pond	Water level (cm)					Salinity level (g/L)					pH					Dissolved O <sub>2</sub> (mg/L)					Transparency (cm)					
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	
AMPH10	25	20	9	0		2.5	3.1	4.9			8.1	8.4	9.2			8	6	11			39	26	30			
BAIBAS	42	37	30	23	5	3.7	4.6	4.9	5.0	7.4	8.5	8.8	9.4	8.0	8.5		14	14	17	14	35	26	29	31	13	
BAIIRI	13	18	12	9	13	2.9	3.2	3.7	5.0	4.0	8.6	8.9	8.8	9.1	9.2		9	3	4	11	15	18	18	13	13	
BAINET	16	21	15	11	16	3.3	3.0	3.1	3.1	2.1	8.3	7.9	7.6	8.0	8.5		15	4	10	15	18	22		22	20	
BAIPES	41	35	27	17	11	11.0	11.7	13.4	15.7	16.6	8.4	7.9	8.1	8.4	8.5		6	8	13	11	17	20	15	13	11	
BRISCO	24	20	8	0		5.6	6.8	13.6			8.1	8.7	8.4			8	8	9			19	15	29			
CDM	30	29	29	25	25	2.5	2.7	3.1	2.9	3.0	8.5	8.9	9.2	9.2	9.6	12	8	17	9	14	6	25	18	31	39	
CERI M	37	31	16	1	0	1.9	2.1	2.8	4.6		8.8	9.2	10.2	10.1		11	15	17	19		25		25	22		
CERI S	32	24	16	2	0	3.1	3.4	4.8	7.4		9.1	9.6	10.6	10.1		12	14	13	19		31		36	27		
FANGOU	21	17	9	0		2.8	3.4	4.8	7.1		8.0	7.7	8.8	9.1			7	10	17		30	26		35		
LAGNAP	7	34	8	15	8	7.7	8.3	11.2	10.7	3.7	8.8	8.3	8.3	9.0	8.6		10	9	11	11	22	12		42	43	
MAB	50	43	29	20	11	4.0	4.3	4.9	5.5	7.5	8.5	9.1	9.9	10.3	10.3	8	7	18	18	17	13	16	34	32	37	
MARCAR	56	48	29	10	-15	2.9	3.6	4.8	7.4	11.1	8.1	8.4	8.0	7.5	9.4		11	11	7	19	30	35		27	28	
MATEMP	30	26	20	13	3	7.5	8.4	11.6	15.4	31.7	8.7	8.8	9.2	9.1	9.0	11	5	16	15	16	5	21	8	8	8	
MONCAN	62	56		65	45	0.6	1.0		1.1	1.2	7.7	7.8		7.9	9.0		16		6	12	26			55	42	
PALBOU	19	40	21	-1	10	0.3	0.3	1.0	1.1	0.9	7.5	7.5	7.8	9.1	7.5		5	7	13	9	20	47		22	36	
PALU N	29	27	26	23	22	2.5	2.2	8.2	2.1	2.4	8.4	8.4	8.3	8.0	8.9	10	9	12	5	12	12	15	23	25	18	
RELO S	34	29	22	15	-1	4.0	4.8	6.1	8.3	10.9	8.6	9.5	10.3	10.1	9.7		16	16	21	13	22		16	23	31	
RELONE	34	30	25	30	21	4.3	5.5	6.7	7.8	11.5	8.3		9.0	8.6	7.9			10	15	9			22	25	31	
RIZET	40	33	26	19	12	2.8	3.1	3.6	4.4	5.7	8.7	9.8	9.1	9.5	9.4	11	8	9	15	10	28	35	30	34	26	
SALINE	43	40	33	26	14	5.8	6.4	7.7	9.1	13.1	8.4	8.3	8.9	8.5	7.9	13	9	11	14	8	23	15	17	21	15	
SAM	24	21	23	17	16	2.1	2.7	2.4	2.3	2.5	8.5	8.1	7.8	7.6	8.0	14	17	9	2	3	26	25	18	26	23	
SARCEL	29	23	17	9	0	3.1	4.1	5.1	6.7		7.6	7.5	7.9	7.7			5	9	10		30			45		
TAMARG	42	37	28	18	0	2.5	2.7	3.3	4.4	8.6	8.8	9.2	10.5	9.3	9.1	12	13	16	9	20	19		30	25	12	
TDCIRR	57	52	41	31	28	13.4	14.9	19.1	21.7	21.3	8.0	8.0	8.0	7.9	8.1		9	6	7	7	23	22	19	13	14	
TDCORS	25	23	22	20	18	2.4	2.5	2.5	2.5	2.0	8.4	7.9	7.8	8.3	8.9	12	5	6	6	11	30	21	33	26	38	
TDH	59	57	52	45	31	7.5	8.3	10.8	11.5	15.5	8.4	8.2	8.9	9.1	8.6	8	5	21	21	13	34.5	21	32	27	36	
TDPELO	56	50	37	20	2	1.2	5.4	1.2	1.3	1.3	7.8	7.9	8.1	8.8	9.6		8	11	9	18	35	32	43	32	31	
TIRA R	75	65	53	41	22	9.1	10.1	11.9	14.9	21.7	8.4	8.6	8.6	8.5	8.6		9	8	9	7	8	29	37	39	29	18
TIRA W	36	33	23	14	6	3.6	4.3	5.6	6.9		8.3	8.1	8.9	8.8		9	7	17	18		23	23	26	29		
TRAFAL	16	11	1		0	4.5	5.1	11.7			9.7	9.8	10.2			11	12	14			23		18			
TRINCA	28	32	26	19	14	1.6	1.1	1.4	1.9	2.6	8.7	8.6	7.6	8.6	8.5	16	12	2	10	8	11		11	16	26	
TRNOUV	51	44	29	8	0	5.9	6.2	7.1	8.8	12.4	7.9	8.3	8.0	8.4	8.3		9	5	14	14	22	28	37	30	17	

Table S3. Abundance of taxa (other than odonates) considered as predators of *Lestes macrostigma* larvae within the animal community samples. See Table S3 for the order of 33 study ponds. Polych. = Polychaeta, Nereid. = Nereidae; Crust. = Crustacea, Palae. = Palaemonidae, *P.clarkii* = *Procambarus clarkii*, *C.aestu.* = *Carcinus aestuarii*, Insect. = Insecta, Nauco. = Naucoridae, Noton. = Notonectidae, Corix. = Corixidae, Gyrin. = Gyridae, Hydro. = Hydrophilidae, Dytisc. = Dytiscidae, lv = larvae, ad = adults.

Pond	Polych.	Crust.	Insect.								
	Nereid.	Palae.	<i>P.clarkii</i>	<i>C.aestu.</i>	Nauco.	Noton.	Corix.	Gyrin. lv	Hydro. lv	Dytisc. lv	Dytisc. ad
BAIIRI	–	–	4	–	1	–	–	–	–	2	3
BAINET	–	–	–	–	2	1	–	–	14	–	12
LAGNAP	–	5	–	1	–	–	–	–	–	–	–
FANGOU	–	–	11	–	–	–	–	–	–	–	39
MAB	–	–	–	–	–	–	–	–	–	10	–
CDM	–	–	–	–	3	–	–	–	–	1	3
PALBOU	–	–	–	–	1	–	–	–	2	13	92
PALU N	–	–	–	–	–	–	–	–	–	2	–
SAM	–	–	–	–	–	–	–	–	–	–	7
TDH	–	–	–	–	–	–	–	1	–	–	9
BRISCO	–	595	–	–	–	–	–	–	–	13	2
TIRA R	8	–	–	–	–	–	–	–	–	–	3
RIZET	–	–	5	–	–	–	–	–	–	4	9
RELONE	–	–	22	–	1	–	–	–	–	–	1
TRAFAL	–	–	–	–	–	–	–	–	–	2	1
TRNOUV	–	–	–	–	–	–	–	–	–	8	5
TDCORS	–	–	–	–	2	–	1	–	1	12	14
MATEMP	–	–	–	–	–	–	–	–	4	14	8
TDPELO	–	–	–	–	–	–	–	–	–	–	4
BAIPES	9	–	–	–	–	–	1	–	–	–	–
MONCAN	–	–	–	–	–	–	–	–	–	–	85
TDCIRR	4	–	–	–	–	–	–	–	–	–	–
TRINCA	–	–	6	–	–	–	–	–	–	–	1
AMPH10	–	–	–	–	–	–	–	–	–	17	5
RELO S	–	–	–	–	–	–	–	–	–	3	3
SALINE	–	–	2	–	–	–	–	–	–	–	–
MARCAR	–	–	1	–	1	–	–	–	–	16	10
TIRA W	–	–	–	–	–	–	–	–	–	23	5
SARCEL	–	–	–	–	1	–	–	3	3	8	9
CERI M	–	–	–	–	–	–	–	–	–	25	46
TAMARG	–	–	–	–	1	1	1	–	–	22	26
CERI S	–	–	–	–	–	–	–	–	–	4	38
BAIBAS	–	–	–	–	–	–	–	–	–	10	7



Table S4. Abundance of odonate taxa within the animal community samples. The 33 study ponds are order according to increasing flooding date of the pond first (expressed as week number, with the weeks of the 1<sup>st</sup> January 2013 and 2014 being respectively 1 and 53), and to increasing *Lestes macrostigma* density then. (*Les. mac.* = *Lestes macrostigma*, *Les. bar.* = *Lestes barbarus*, *Les. spo.* = *Lestes sponsa*, *Sym. fus.* = *Sympecma fusca*, *Isc. ele.* = *Ischnura elegans*, *Aesh.* = Aeshnidae, *Cro. ery.* = *Crocothemis erythraea*, *Les. Symp.* = *Sympetrum*, *Orth.* = *Orthetrum*).

Flooding date	Pond	<i>Les. mac.</i>	<i>Les. bar.</i>	<i>Les. spo.</i>	<i>Sym. fus.</i>	<i>Isc. ele.</i>	Aesh. (total)	bigger Aesh. (HW > 4.5 mm)	<i>Cro. ery.</i> & <i>Symp. spp.</i>	<i>Orth. spp.</i>
32	BAIIRI	–	–	–	–	98	–	–	–	–
	BAINET	–	–	–	–	121	–	–	7	2
37	LAGNAP	–	–	–	–	–	–	–	–	1
38	FANGOU	–	–	–	–	–	33	27	2	–
40	MAB	22	8	5	–	–	–	–	–	–
	CDM	5	–	7	–	5	17	1	8	–
42	PALBOU	–	–	1	–	6	1	1	–	–
43	PALU N	1	–	3	–	5	2	2	–	–
47	SAM	111	–	6	–	–	3	–	–	–
	TDH	92	–	–	–	202	–	–	–	–
	BRISCO	59	–	–	–	–	1	–	14	–
	TIRA R	40	–	–	–	–	–	–	–	–
	RIZET	39	40	50	–	–	5	5	–	–
	RELONE	27	12	–	–	6	7	1	16	1
	TRAFAL	19	6	–	–	–	–	–	1	–
	TRNOUV	10	53	–	–	–	2	2	3	–
	TDCORS	6	–	6	–	1	7	–	2	–
	MATEMP	3	–	1	–	1	–	–	–	–
	TDPELO	1	63	–	–	–	3	3	7	–
	BAIPES	–	–	–	–	–	–	–	–	–
	MONCAN	–	–	–	–	7	6	6	–	–
	TDCIRR	–	–	–	–	–	–	–	–	–
	TRINCA	–	–	1	40	–	–	–	2	–
51	AMPH10	69	–	–	–	–	1	–	5	–
54	RELO S	7	–	1	–	–	–	–	–	–
	SALINE	–	–	–	–	–	–	–	–	–
55	MARCAR	196	38	–	–	–	–	–	4	–
	TIRA W	71	–	–	–	–	–	–	42	–
	SARCEL	24	100	–	–	–	–	–	15	–
	CERI M	20	25	–	–	–	–	–	–	–
	TAMARG	19	15	–	–	–	8	–	1	–
	CERI S	9	3	–	–	–	–	–	1	–
	BAIBAS	2	–	–	–	–	–	–	4	–

## Chapter 4

### Oviposition plant choice maximizes offspring fitness in an aquatic predatory insect

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Slightly adapted

#### *Abstract*

Evidence for the adaptive value of oviposition site selection in terms of increased offspring fitness is rare in predatory insects. We tested this in the damselfly *Lestes macrostigma* that prefers the plant *Bolboschoenus maritimus*. We carried out two experiments with shoots containing eggs: we flooded (1) some shoots of the same type (i.e. combination of species and desiccation state) at different dates and (2) different shoot types at the same date. Earlier flooding increased hatching success. Because *B. maritimus* grows in deeper parts of temporary ponds, it is flooded before other plants after the drought season, suggesting that adult oviposition site selection is driven by lower egg desiccation risk. Independently of flooding date, hatching success was higher and larvae hatched earlier when eggs were laid in *B. maritimus*. Faster embryonic development enhances chances to complete larval development before pond desiccation and reduces costs associated with time stress. Offspring higher fitness was more constant between shoots of *B. maritimus* compared to the other types of shoot, suggesting that laying eggs in one shoot of this plant leads offspring to high fitness more surely. Our results indicate that adults choose oviposition plants maximizing offspring fitness (higher hatching success and faster embryonic development).

## Introduction

In organisms using an opportunistic reproductive strategy (*r*-selection), such as insects that lay large egg clutches, oviposition site selection is key to increase offspring survival, and therefore can be regarded as a form of parental care (Janz 2002; but see García-Robledo & Horvitz 2012). Indeed, oviposition site characteristics such as abiotic conditions, vegetation abundance or structure and the presence of natural enemies can have dramatic impact on egg and larval survival in various insect orders (e.g. Rausher 1979; Sweeney & Quiring 1998; Pasquier-Barre et al. 2001; Hirayama & Kasuya 2009; Knorp & Dorn 2016). Therefore, adult selection of oviposition sites is of paramount importance for population dynamics. Oviposition site selection in relation to plant features has been widely studied in herbivorous insects where it is mainly directed to maximize food for the offspring (Tilmon 2008). These plant features impacting the offspring can vary at the plant inter-specific level but also the plant inter- and intra-individual levels (Rausher 1979; Pasquier-Barre et al. 2001). In predatory insects, the vegetation characteristics of the mesohabitat may drive specific oviposition frequency or larval survival, and therefore species assemblages (Knorp & Dorn 2016; French & McCauley 2018). On the contrary, little is known on the adaptive value of oviposition microhabitat selection in predatory insects that lay eggs within plants. In these species the oviposition plant is not used a food source. Hence, a question is whether there is any benefit for the offspring of such insects that their adults prefer some plant species or individuals to lay their eggs?

Within the order of Odonata, predatory insects with an aquatic larval stage and terrestrial adult stage (Stoks & Cordoba-Aguilar 2012), many species use an endophytic mode of oviposition whereby females insert their eggs directly into the plant substrate using an ovipositor (Corbet 2004). For many dragonflies, vegetation is thought to be an important macro-habitat factor used to select oviposition sites that maximize the survival requirements of the offspring (Buskirk & Sherman 1985; Martens 2001; Srivastava et al. 2005). At the micro-habitat scale, endophytic oviposition is a million-year-old reproductive strategy thought to be an effective mechanism against desiccation and predation of eggs (Corbet 1956a; Béthoux et al. 2004; Goula 2008). However, evidence for a link between oviposition site preference of adults and offspring survival is scarce (but see Grunert 1995; Siva-Jothy et al. 1995).

The damselfly *Lestes macrostigma* (Eversmann, 1836) (Odonata: Lestidae) reproduces in temporary brackish waters (Boudot & Raab 2015). This species shows great inter-annual variation in population abundance and site occupancy (Borisov 2005; Lambret et al. 2009; Florencio & Diaz-Paniagua 2012) which contributes to its former threatened and now 'Near Threatened' conservation status within the EU (Kalkman et al. 2010; G. de Knijf pers. comm. 2024). To lay eggs, *L. macrostigma* can use living or dead shoots of several plant species but it prefers the living shoots of the Sea club-rush *Bolboschoenus maritimus* (L.) Palla (i.e. stems covered by leaves) over other combinations of plant

species and desiccation state (Lambret et al. 2015a, 2015b). Specific plant preference can be found in other odonates such as the damselfly *Ischnura kellicotti* Williamson, 1898 associated with *Nymphaea* spp. and *Brasenia* spp. lily pads or the dragonfly *Aeshna viridis* Eversmann, 1836 requiring the hydrophyte *Stratiotes aloides* (e.g. Paulson 2012, 2017; Suhonen et al. 2013). As a second choice *L. macrostigma* prefers *Juncus* shoots (i.e. stems and leaves, which are indistinctly used) for oviposition where it favours dead shoots over living shoots (Lambret et al. 2015a, 2015b). These preferences consist especially in a higher oviposition rate (i.e. the number of eggs laid per minute) in shoots of *B. maritimus* and dead shoots of *Juncus maritimus* Lam. (Lambret et al. 2015b). But yet to date, whether *L. macrostigma* offspring benefits from adults' preferences remains unknown. Species inhabiting temporary ponds often show winter egg diapause and fast larval growth (e.g. Corbet 1956a; Carchini et al. 2007; De Block et al. 2008). Accordingly, the univoltine life cycle of *L. macrostigma* is characterised by eggs laid during late spring with the start of embryonic development during the drought season; thereafter eggs aestivate and overwinter inside the oviposition plants at an early embryonic development stage and then hatch early at the beginning of the next spring (Lambret et al. 2017). Wetting or water temperature elevation are important changes in environment conditions which trigger egg hatching and influence hatching success in temporary pond dragonflies (Sawchyn & Gillot 1974a; Schiel & Buchwald 2015a; Lambret et al. 2017). When hatching is triggered late in the season, temporary pond larvae experience a strong time constraint they can cope with by fastening their development; but this may have dramatic costs during the larval and adult stages, such as immune reduction, size at emergence, mating success (De Block & Stoks 2004, 2005a; Stoks et al. 2006). A noteworthy difference between *B. maritimus* and *Juncus* spp. shoots is their position in temporary ponds: *B. maritimus* grows in the deeper parts of the pond so that the flooding of eggs laid in *B. maritimus* happens earlier compared to the other types of shoot *L. macrostigma* is using during oviposition (Grillas et al. 2004).

In this work, we investigated if the oviposition plant selection of *L. macrostigma* is adaptive in terms of offspring fitness. We tested three hypotheses: (1) whether hatching success is higher when egg clutches are flooded earlier after the drought period, (2) whether hatching success is higher and egg development time shorter when eggs are laid in the types of shoots adults prefer (i.e. living shoots of *B. maritimus* and dead shoots of *J. maritimus*), and (3) whether inter-shoot variance of hatching success and egg development time is lower within a given type of shoot, making oviposition in this substrate less haphazard for the offspring. We tested these hypotheses by carrying out two experiments where shoots containing egg clutches were monitored individually: (1) we flooded some shoots of the same type at different dates and (2) we flooded different types of shoot at the same date.

## Methods

### *Egg clutch sampling and plant shoot types*

All tests were done with *L. macrostigma* egg clutches sampled in several water bodies within the Marais du Vigueirat National Nature Reserve (Baisse des Marcells: 43°29'27"N | 04°48'22"E; Trou du Héron: 43°30'51"N | 04°47'01"E) and the Tour du Valat Regional Nature Reserve (Tirasses ouest: 43°29'43"N | 04°38'36"E; Saline: 43°29'10"N | 04°38'44"E). These two protected areas are located ca. 5 km apart in the Rhône delta (southern France).

We searched in the field for shoots with eggs of four different plant species: *B. maritimus*, *J. maritimus*, *J. acutus* L., and *Phragmites australis* (Cav.) Steud. Shoots bearing egg clutches, if not dead yet at the moment of oviposition, usually die during the drought period. Depending on their life-span, *J. maritimus* shoots at the moment of oviposition are a mixture of living and dead shoots. To favor natural drying of the shoots, we marked shoots with egg clutches and collected them after the oviposition period. Collected plants were categorized in one of three groups: (1) dead before oviposition (dead), (2) drying out between oviposition and collection (dying), and (3) still living upon collection (living). Shoots of *B. maritimus* were all of the (dying) condition. Shoots of *J. maritimus* were distributed in all three shoot conditions. *Lestes macrostigma* only uses dead shoots when laying eggs in *Juncus acutus* and *P. australis* so that these shoots were all of the (dead) condition.

### *Experiment 1: Influence of flooding on egg hatching success*

In a first experiment, we tested for the effect of the occurrence of flooding and the date of flooding on egg hatching success. For this, dead shoots of *J. maritimus* with *L. macrostigma* eggs were collected during June 2013 and kept outdoors until the start of the experiment on 20 November 2013. Then, each shoot was placed in or above (see treatments) a bucket which was 30 × 35 cm (height × diameter) and half-filled with fresh water. Buckets were kept outdoors at the Tour du Valat Research Institute.

Four flooding treatments were applied, each with five replicates: (1) shoots submerged on 20 November 2013 (autumn flooding, corresponding to the common natural hydroperiod), (2) shoots submerged on 20 February 2014 (winter flooding, corresponding to a more rare natural hydroperiod), (3) shoots kept above the water and sprayed with fresh water once a week in order to mimic incomplete flooding with rainfall (rain treatment), and (4) shoots kept as in (3) but with no spraying (dry treatment). As no larvae had hatched from treatments (3) and (4) at the end of the natural hatching period, which lasts in the Camargue from ca. 15 March to ca 10 April (Lambret et al. 2017), shoots of these treatments were combined, and evenly distributed in two additional treatments also with five replicates: shoots submerged on (5) 10 April (early spring flooding) and (6) 30 May (late spring

flooding). Buckets were placed into tanks to prevent spilling and left in open air for the whole duration of the experiment. They were subject to natural weather conditions and the position of each bucket in its tank was randomly changed everyday. Water within the bucket was renewed approximately every two weeks.

#### *Experiment 2: Influence of shoot on larval hatching success and hatching date*

In a second experiment, we tested for the effect of type of shoot and of the shoot itself (i.e. among shoots of a same type) on larval hatching success and hatching date. Shoots of six different plant types with *L. macrostigma* egg clutches were collected during June 2014 (Fig. 4.1a–f): (1) *B. maritimus* (dying), (2) *J. acutus* (dead), (3) *J. maritimus* (dead), (4) *J. maritimus* (dying), (5) *J. maritimus* (living) and (6) *P. australis* (dead). To reduce desiccation risk, shoots were kept indoors during summer and early autumn. Afterwards, based on the results of the first experiment, they were submerged on 29 November 2014. Six shoots, i.e. six replicates, of each of the six plant types were each placed into different buckets (one shoot per bucket) that were half-filled with fresh water (36 buckets in total). These 36 buckets were placed into six tanks, with each tank containing one shoot (i.e. one bucket) of each of the six types (Fig. 4.1g). Buckets conditions and water renewal were similar as previous experiment.

#### *Monitoring of temperature and photoperiod*

Mean daily air temperature and photoperiod were obtained from the meteorological station of 'Arles-Valat' (Météo France, n°13004003, 43°30'36"N | 4°41'38"E) at 2 km from the study site. During the first experiment, as the treatments differed in the timing, water temperature varied among treatments and was not recorded. During the second experiment, water temperature was measured every week (mid-afternoon) from 29 November 2014 until 22 April 2015, at which time the hatching had ended (see below). To assess if water temperature varied significantly between the two experiments, we tested whether water temperature depended on air temperature.

#### *Determination of hatching success and hatching date*

Based on Matushkina & Lambret (2011) and Lambret et al. (2017), we assumed that one full incision created by the female when inserting entirely the ovipositor into the substrate corresponded to one egg and that a smaller incision testified of a failure in laying one egg. Hence, we counted the number of full incisions to quantify the number of eggs in each shoot. From the start of the experiments until



Figure 4.1 – Different plant types used by *Lestes macrostigma* to lay its eggs: (a) dying *Bolboschoenus maritimus* (a), dead *Juncus acutus* (b), dead *Phragmites australis* (c), and – same picture, with each letter on corresponding type of shoot – *Juncus maritimus* dead (d), dying (e) or living (f).

the first larva was found in the water, buckets were monitored twice per week. From then onwards, larvae were counted and removed from each bucket every two days. Hatching was considered to have finished after three consecutive counts of zero larvae in all buckets, except with treatments (3) and (4) (see above) and for treatment (6) for which larvae were searched on six occasions between 20 June – 15 July 2014.

*Data analysis*

The correlation between mean air temperatures and bucket mean water temperatures was tested using a Spearman correlation test. We tested the differences between flooding treatments and plant shoot types using Generalized Linear Mixed Models (GLMM). GLMM do not compute mean values or frequencies but predict them from the data (Bolker 2008). Results are therefore mean values or frequencies predicted by the models. A 'shoot' random effect was included in all models to take into account that several eggs were in the same shoot. Hatching success (proportion of eggs that hatched per shoot) was analyzed using a binomial error structure (Zuur et al. 2009). Hatching dates were expressed as log (Julian dates), with day one being 1 January, and analyzed using a Gaussian error structure. We checked model assumptions (the normality, the heteroscedasticity and independence of the residuals) following Zuur et al. (2009). Significances were tested by analysis of deviance. Contrast analyses were performed in order to assess which shoot types differed from each other.

To test the inter-shoot variance of hatching success and egg development time regarding shoot types, we assessed the effect of the 'shoot' random effect. This random effect could not be set as a fixed factor because 'shoot' is typically unpredictable, therefore random. First, we compared the predicted values of our GLMM to these of a Generalized Linear Models (GLM) i.e. not including 'shoot' as random effect. For so, we computed a variation rate (VR) as following:

$$VR = \left| \frac{PV(GLMM) - PV(GLM)}{PV(GLM)} \right|$$

where PV is the predicted value – of hatching rate or hatching date – of the corresponding model; VR is given as a percentage. Second, we computed the exact value of each of the random effect (i.e. the effect of each shoot) using 1,000 posterior simulations (Gelman & Hill 2006). We then compared the standard deviation SD of computed random effects with respect to types of shoot.

Results are given as means or frequencies with their lower and upper 95% confidence intervals (unless otherwise stated). All analyses were performed with R 3.3.2 (R Core Team 2020), more specifically with libraries 'lme4' (Bates et al. 2019), 'nlme' (Pinheiro et al. 2017) and 'MASS' (Ripley et al. 2018) to compute predicted values and perform general tests and contrast analyses, 'effects' (Fox et al. 2016) to compute predicted values 95% confidence intervals, and 'merTools' (Knowles & Frederick 2016) to assess random effect contribution to the predicted values and the constancy of its effect between shoots of the same type.



## Results

We studied 20 shoots containing 1,468 eggs in experiment 1 (mean  $\pm$  SD =  $73.4 \pm 44.4$  eggs, min = 13, max = 193) and 36 shoots containing 3,908 eggs in experiment 2 (mean  $\pm$  SD =  $109.1 \pm 92.2$  eggs, min = 19, max = 387).

## Temperature

The air temperature ranged from 5.34 °C in November 2013 to 21.94 °C in July 2014, and from 6.51 °C in December 2014 to 14.94 °C in April 2015 (Fig. 4.2; Tab. 4.1). Mean daily air temperature was significantly higher in 2013–2014 than in 2014–2015 (Friedman test,  $Q = 23.76$ ,  $df = 1$ ,  $p < 0.001$ ): this was especially due to the differences observed in January, February and April (Tab. 4.1). Water temperature correlated positively with air temperature (Spearman correlation test:  $r = 0.839$ ,  $n_{\text{pairs}} = 20$ ,  $p < 0.001$ ; Fig. 4.1), suggesting that water temperature was also higher in 2013–2014 than in 2014–2015. Water temperatures in the buckets in 2014–2015 did not differ between the shoot types (GLMM with paired dates as correlation structure:  $t \in [-0.069; 0.297]$ , each  $df = 30$ ,  $p \in [0.768; 0.945]$ ).

## Influence of the date of flooding on hatching success

No larvae hatched in the treatments where shoots were not submerged, i.e. the ‘rain’ and ‘dry’ treatments, and in the ‘late spring flooding’ treatment. In the other treatments where shoots were submerged, the hatching success differed significantly with respect to the date of flooding (GLMM:  $\chi^2 = 8.736$ ,  $df = 2$ ,  $p = 0.012$ ; Fig. 4.3). The hatching success was higher when flooding occurred in the ‘autumn’ than in the ‘winter’ ( $z = -2.114$ ,  $df = 1$ ,  $p = 0.035$ ) and ‘early spring’ ( $z = -3.417$ ,  $df = 1$ ,  $p < 0.001$ ). Hatching success did not differ between the ‘winter flooding’ and the ‘early spring flooding’ treatments ( $z = -1.325$ ,  $df = 1$ ,  $p = 0.185$ ).

Table 4.1 – Friedman tests per paired fortnight for the mean daily air temperature between the two experiments (experiment 1: 2013–14 and experiment 2: 2014–15) of egg hatching in *Lestes macrostigma*. Coding of significance levels: ns  $p > 0.1$ ; ■  $p \leq 0.1$ ; \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ .

Month	Fortnight	Year	Mean $\pm$ SD (n)	Friedman test
Dec	1	2013–14	6.9 $\pm$ 2.0 (15)	Q = 5.400, $p = 0.020$ *
		2014–15	8.6 $\pm$ 2.5 (15)	
	2	2013–14	9.6 $\pm$ 2.6 (16)	Q = 2.250, $p = 0.134$ ns
		2014–15	7.7 $\pm$ 3.3 (16)	
Jan	1	2013–14	10.3 $\pm$ 1.9 (15)	Q = 3.267, $p = 0.071$ ■
		2014–15	8.4 $\pm$ 3.1 (15)	
	2	2013–14	8.4 $\pm$ 2.4 (16)	Q = 5.400, $p = 0.020$ *
		2014–15	6.5 $\pm$ 1.9 (16)	
Feb	1	2013–14	9.5 $\pm$ 1.9 (15)	Q = 14.000, $p < 0.001$ ***
		2014–15	4.9 $\pm$ 2.6 (15)	
	2	2013–14	9.7 $\pm$ 1.2 (13)	Q = 9.308, $p = 0.002$ **
		2014–15	7.9 $\pm$ 1.1 (13)	
Mar	1	2013–14	10.4 $\pm$ 1.4 (15)	Q = 0.286, $p = 0.593$ ns
		2014–15	10.0 $\pm$ 2.2 (15)	
	2	2013–14	12.3 $\pm$ 2.1 (16)	Q = 0.067, $p = 0.796$ ns
		2014–15	12.2 $\pm$ 2.3 (16)	
Apr	1	2013–14	15.7 $\pm$ 1.5 (15)	Q = 15.000, $p < 0.001$ ***
		2014–15	12.4 $\pm$ 1.8 (15)	

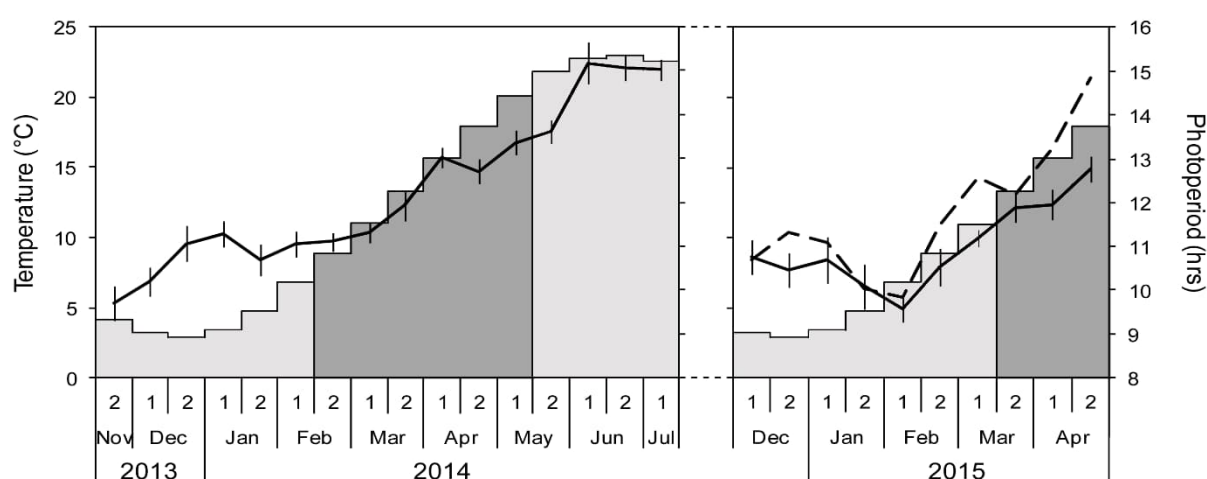


Figure 4.2 – Natural outdoor fortnight temperature (primary axis) and photoperiod (secondary axis) when *Lestes macrostigma* eggs were into the water during the 2013–2014 and 2014–2015 experiments. Temperature curves indicate the mean daily air temperature (full line with 95% confidence intervals) and afternoon water temperature (dotted line). The dark grey shading indicates the hatching period.

*Influence of the shoot type on egg hatching success and hatching date*

Egg hatching success differed significantly among shoot types (GLMM:  $\chi^2 = 12.05$ ,  $df = 5$ ,  $p = 0.034$ ; Fig. 4.4). Hatching success was higher in *B. maritimus* than in *J. maritimus* (living) ( $z = -3.003$ ,  $df = 1$ ,  $p = 0.003$ ) and *P. australis* ( $z = -1.993$ ,  $df = 1$ ,  $p = 0.046$ ), and marginally higher than in *J. maritimus* (dead) ( $z = -1.874$ ,  $df = 1$ ,  $p = 0.061$ ) (Tab. 4.2). The hatching success in *J. maritimus* (living) was the lowest and differed significantly from that of eggs laid in shoots of *B. maritimus* (see above) and of *J. maritimus* (dying) ( $z = 2.442$ ,  $df = 1$ ,  $p = 0.015$ ) and of *J. acutus* ( $z = 2.652$ ,  $df = 1$ ,  $p = 0.008$ ) (Tab. 4.2).

The egg hatching date differed significantly among shoot types (GLMM:  $\chi^2 = 27.69$ ,  $df = 5$ ,  $p < 0.001$ ; Fig. 4.5). Larvae hatched the earliest from shoots of *B. maritimus* (Fig. 4.5). The hatching date was significantly earlier in *B. maritimus* than in *J. maritimus* (dead) ( $t = 2.593$ ,  $df = 1$ ,  $p = 0.015$ ) or (dying) ( $t = 3.223$ ,  $df = 1$ ,  $p = 0.003$ ) or (living> living) ( $t = 5.701$ ,  $df = 1$ ,  $p < 0.001$ ) and in *P. australis* ( $t = 3.043$ ,  $df = 1$ ,  $p = 0.005$ ) (Tab. 4.3). The mean hatching date in *B. maritimus* shoots was also earlier than that of eggs in shoots of *J. acutus* although with low significance ( $t = 1.759$ ,  $df = 1$ ,  $p = 0.089$ ).

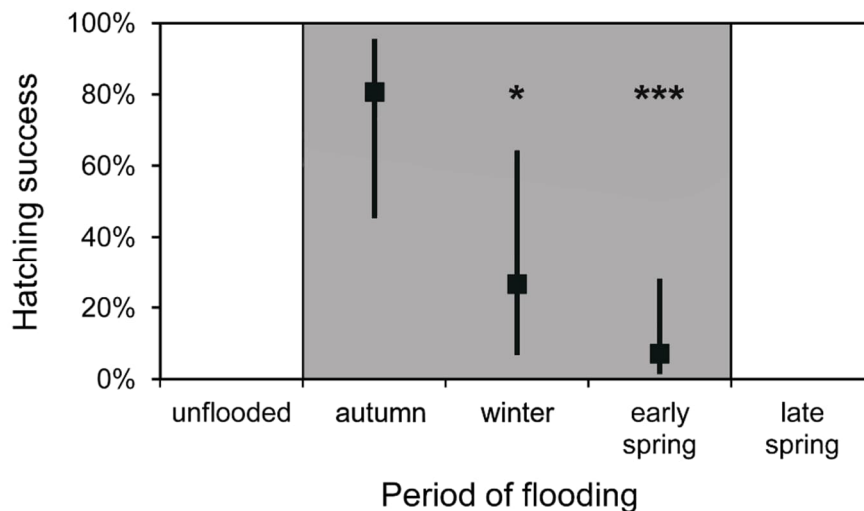


Figure 4.3 – Hatching success (means with 95% confidence intervals) of *Lestes macrostigma* eggs for the different periods of flooding. For example, a hatching success of ca. 80% for autumn means that ca. 80% of the eggs laid in the shoots flooded in autumn hatched with success. Significance of the difference with the hatching success of eggs flooded in autumn is indicated by \*  $p \leq 0.05$ ; \*\*\*  $p \leq 0.001$ . As no eggs hatched from unflooded shoots and from shoots flooded in late springs, these two treatments were not included in the analysis.

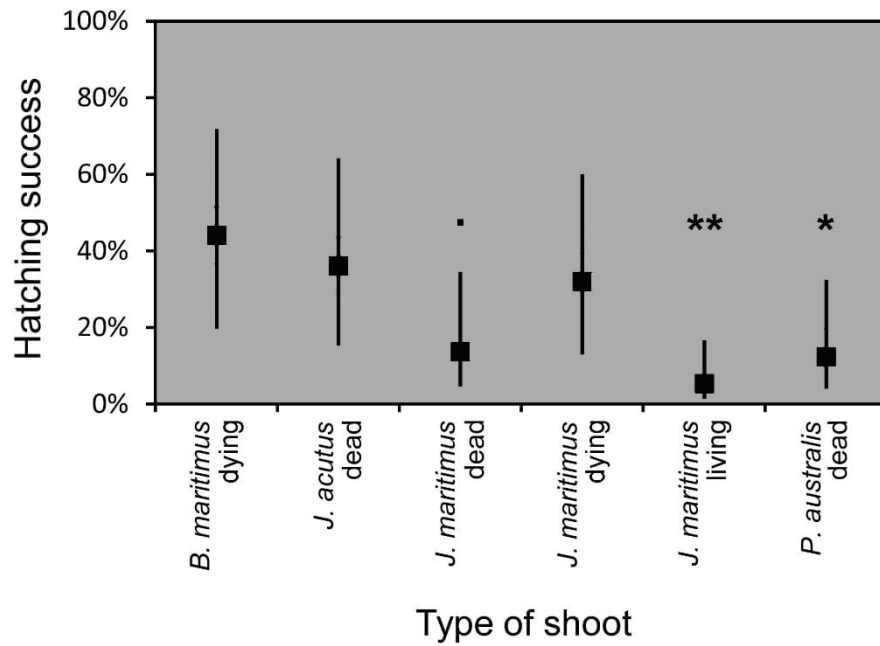


Figure 4.4 – Hatching success (means with 95% confidence intervals) of *Lestes macrostigma* eggs for the different shoot types (see Fig. 4.1 for the difference between living, dying and dead shoots, especially in *Juncus maritimus*). For example, a hatching success of ca. 40% for ‘*B. maritimus* dying’ means that ca. 40% of the eggs laid in this shoot type hatched with success. Shoot types are a combination of the plant species and the condition of the shoot (living, dying or dead). Significance level of the difference with the hatching success of eggs laid in *Bolboschoenus maritimus* is indicated as: ■  $p \leq 0.1$ ; \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ .

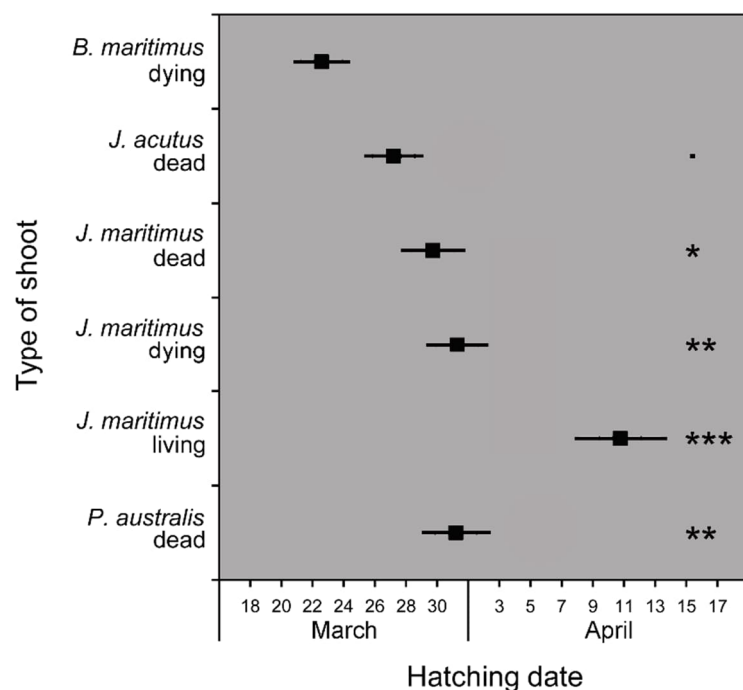


Figure 4.5 – Mean hatching date (with 95% confidence intervals) of *Lestes macrostigma* eggs for the different shoot types. Significance level of difference from eggs laid in *Bolboschoenus maritimus* is indicated as: ■  $p \leq 0.1$ ; \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ .

Table 4.2 – Contrast analysis for the hatching success between the different shoot types used by *Lestes macrostigma* as oviposition substrate. Coding of significance levels as in Table 4.1.

	<i>B. maritimus</i> (dying)	<i>J. acutus</i> (dead)	<i>J. maritimus</i> (dead)	<i>J. maritimus</i> (dying)	<i>J. maritimus</i> (living)	<i>P. australis</i> (dead)
<i>B. maritimus</i> (dying)		$z = 0.395$ $p = 0.693$	$z = 1.874$ $p = 0.061$	$z = 0.614$ $p = 0.540$	$z = 3.003$ $p = 0.003$	$z = 1.993$ $p = 0.046$
<i>J. acutus</i> (dead)	ns		$z = 1.499$ $p = 0.134$	$z = 0.221$ $p = 0.825$	$z = 2.652$ $p = 0.008$	$z = 1.621$ $p = 0.105$
<i>J. maritimus</i> (dead)	▪	ns		$z = 1.283$ $p = 0.199$	$z = 1.214$ $p = 0.225$	$z = 0.143$ $p = 0.886$
<i>J. maritimus</i> (dying)	ns	ns	ns		$z = 2.446$ $p = 0.014$	$z = 1.409$ $p = 0.159$
<i>J. maritimus</i> (living)	**	**	ns	*		$z = 1.060$ $p = 0.289$
<i>P. australis</i> (dead)	*	ns	ns	ns	ns	

Table 4.3 – Contrast analysis for the hatching date between the different shoot types used by *Lestes macrostigma* as oviposition substrate. Coding of significance levels as in Table 4.1.

	<i>B. maritimus</i> (dying)	<i>J. acutus</i> (dead)	<i>J. maritimus</i> (dead)	<i>J. maritimus</i> (dying)	<i>J. maritimus</i> (living)	<i>P. australis</i> (dead)
<i>B. maritimus</i> (dying)		$t = 1.759$ $p = 0.089$	$t = 2.593$ $p = 0.015$	$t = 3.223$ $p = 0.003$	$t = 5.701$ $p < 0.001$	$t = 3.043$ $p = 0.005$
<i>J. acutus</i> (dead)	▪		$t = 0.894$ $p = 0.379$	$t = 1.478$ $p = 0.150$	$t = 4.232$ $p < 0.001$	$t = 1.380$ $p = 0.178$
<i>J. maritimus</i> (dead)	*	ns		$t = 0.541$ $p = 0.593$	$t = 3.374$ $p = 0.002$	$t = 0.490$ $p = 0.628$
<i>J. maritimus</i> (dying)	**	ns	ns		$t = 2.973$ $p = 0.006$	$t = 0.028$ $p = 0.978$
<i>J. maritimus</i> (living)	***	***	**	**		$t = 2.893$ $p = 0.007$
<i>P. australis</i> (dead)	**	ns	ns	ns	**	

### Influence of the shoot itself on egg hatching success and hatching date

The inclusion in our models of 'shoot' as random effect changed the predicted hatching success of 'autumn', 'winter' and 'early spring' flooding by 26.2, -12.2 and 32.2%, respectively. Also, depending on the type of shoot, the predicted hatching success changed up to 59.4% (Tab. 4.4). These substantial values indicate that variation in egg hatching among shoots of a same type was considerable. However, the VR values in *B. maritimus* did not exceed 9.0%. 'Shoot' random effect had a much lower effect, not to say negligible, on the computing of predicted hatching dates, since VR ranged from 0.2 to 3.4%. Yet, second lowest VR was observed in *B. maritimus* (Tab. 4.4).

'Shoot' random effect had a lower variance in 'early spring' flooding than in 'autumn' and 'winter' flooding (SD: 1.02, 1.94 and 2.19, respectively). This indicates that a long period of drought has a deleterious effect on hatching success in most of the shoots rather than in few of them. Regarding the type of shoot, random effect variance was especially lower in *B. maritimus* for both hatching success and hatching rate (Tab. 4.3; Fig. 4.6). The lower VR and SD values in the preferred oviposition plant *B. maritimus* mean that inter-shoot hatching success and hatching date are less susceptible to variability in this plant substrate compared to other shoot types.

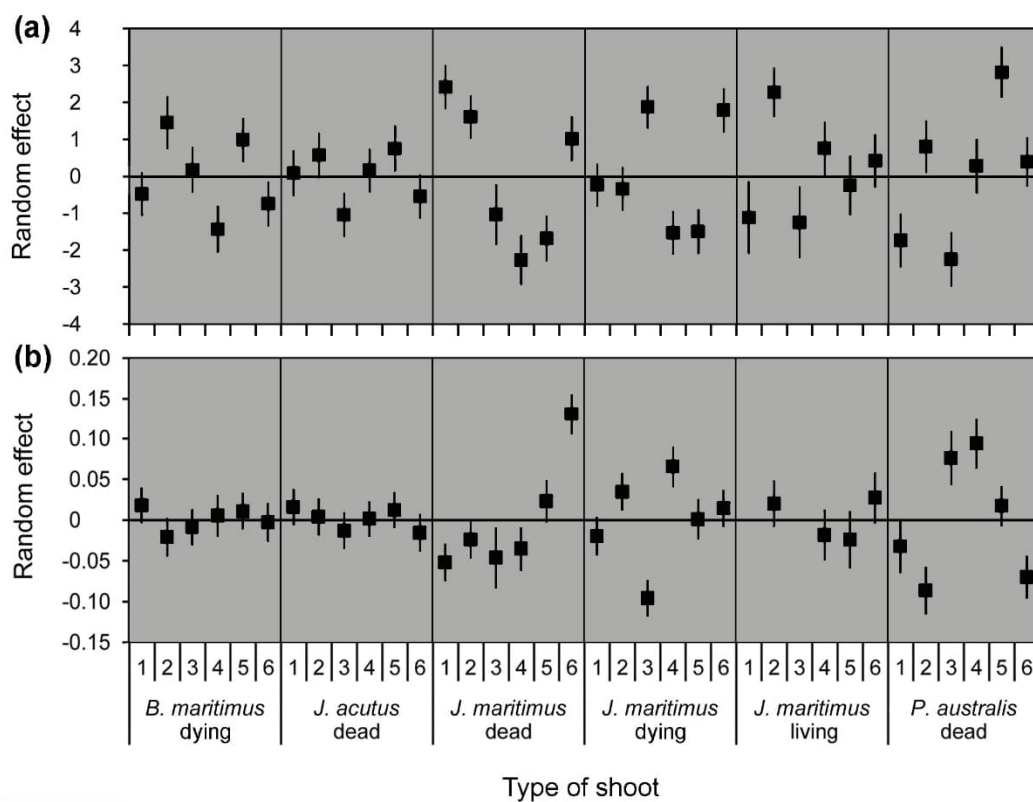


Figure 4.6 – (a) Hatching success and (b) hatching date 'shoot' random effects computed from 1,000 posterior simulations for each shoot of each type (mean  $\pm$  SD).

Table 4.4 – Hatching success and hatching date predictions by GLM and GLMM – the latter including ‘shoot’ as random effect –, variation rate (VR) to which this random effect influenced the predictions and standard deviation (SD) of the random effect with respect to types of shoot.

	Hatching success				Hatching date			
	GLM	GLMM	VR	SD	GLM	GLMM	VR	SD
<i>B. maritimus</i> (dying)	0.41	0.44	9.0%	1.09	82	82	0.3%	0.01
<i>J. acutus</i> (dead)	0.44	0.36	17.6%	0.67	86	86	0.2%	0.01
<i>J. maritimus</i> (dead)	0.27	0.14	48.5%	1.91	87	89	2.5%	0.07
<i>J. maritimus</i> (dying)	0.43	0.32	25.2%	1.50	87	90	3.4%	0.06
<i>J. maritimus</i> (living)	0.13	0.05	59.4%	1.31	102	101	0.9%	0.03
<i>P. australis</i> (dead)	0.13	0.12	4.4%	1.83	90	90	0.4%	0.08

## Discussion

Higher temperatures are associated with faster egg development across many insect groups that undergo a diapause (Rueda et al. 1990; Arrese & Soulages 2010). This certainly explains the ca. one month earlier hatching in 2014 (20 February) than in 2015 (20 March). Photoperiod may also be important in stimulating production of growth hormones regulating embryonic development (Adkisson 1964). We here found a minimal threshold for egg hatching in *L. macrostigma* of ca. 10.7 h daylight (corresponding with 20 February; Fig. 4.2) which is below previous findings (12.8 h daylight; Lambret et al. 2017).

Besides, no eggs hatched in the ‘rain’ and the ‘dry’ treatments; yet, some of these eggs hatched when transferred to the ‘late spring’ treatment. This demonstrates that submersion is necessary for egg hatching in *L. macrostigma*. Larvae did not hatch immediately when eggs were submerged. Instead, with early spring flooding (10 April 2014), a period during which water temperatures were above 12 °C i.e. the threshold expected to trigger hatching in this species (Schiel & Buchwald 2015a; Lambret et al. 2017; Fig. 4.2), first larvae hatched 20 days after immersion. This duration of post-diapause egg development is in accordance with previous findings (Aguesse 1961; Martynov & Martynov 2007) and in line with Sawchyn & Gillott (1974a) who showed that wetting together with increasing temperature can trigger post-diapause egg development in *Lestes*. We found that some eggs are able to hatch even when flooding period is as late as early spring. *Lestes macrostigma* larvae

are therefore able to postpone their hatching for some weeks; a one year shift, seems, however impossible (Schiel & Buchwald 2015a). We also show that the sooner the flooding the higher the hatching success. Early flooding of *L. macrostigma* temporary ponds is therefore important to reduce desiccation risk and egg mortality.

#### *The adaptive value of oviposition substrate preference*

While we here documented successful hatching of *L. macrostigma* larvae from eggs laid in several plant species, our results indicate the adaptive value of laying eggs in *B. maritimus*, the oviposition substrate adults prefer (Lambret et al. 2015a, 2015b). First, *B. maritimus* grows in deeper water than *J. maritimus* and *J. acutus* and therefore undergoes a shorter desiccation period (Aguesse 1961; Grillas et al. 2004). *Lestes macrostigma* prefers to oviposit close to the water (Florencio & Díaz-Paniagua 2012). Hence, Lambret et al. (2015b) hypothesized that such adult behaviour and preference for *B. maritimus* increase the chance of the egg clutches to be flooded early in temporary ponds. Laying eggs in a such substrate is also a relevant reproductive strategy considering the negative effect of low winter temperatures on egg survival since winter water temperature are usually above winter air temperature, and eggs are thereby more sheltered in the water than in the air (Sawchyn & Gillott 1974a; Lambret et al. 2017). Second, egg hatching success was highest in *B. maritimus*, meaning that laying eggs in this type of shoot directly enhance the reproductive success. Yet, we here did not assess the relative importance of each of these two mechanisms and our study does not allow to definitely disentangle what is the nature of the adults' preference.

Third, larvae hatched earliest in *B. maritimus*, indicating a faster egg development. This occurred despite water temperatures did not differ between shoot types. The faster egg development and earlier hatching of *L. macrostigma* in *B. maritimus* enhance the chance of the offspring to relax time stress by providing a longer growth season before pond drying. This reduces the need to increase foraging activity and growth rate and the associated costs in terms of e.g. a lower immune function or a lower mating success (De Block & Stoks 2005a; Stoks et al. 2006). This also creates a benefit in terms of a reduced predation risk from conspecific hatching from other plant species (*Lestes* larvae are cannibalistic; De Block & Stoks 2004) and from congeneric species hatching earlier in average (Schiel & Buchwald 2015a, 2015b). However, accelerated development time in lested larvae lead to emergence at a smaller size or lower body mass (e.g. De Block & Stoks 2003). Following the same mechanism, a shorter embryonic development time may have some fitness costs in the larval and/or the adult stages. The differences in egg survival and development duration among plant species may be due to the surrounding micro-environment provided by the plants: *B. maritimus* shows a



macrofoam-like organisation of tissues (aerenchyma) with aeriferous cavities; *Juncus* spp. and *Phragmites australis* instead have a microfoam-like organisation (Matushkina et al. 2016). We hypothesize that these large aeriferous cavities could enhance egg oxygenation (Corbet 2004) and/or better isolate the egg from extreme temperatures. Repeating our experiments with other plant species used by *L. macrostigma* that share these cavities (e.g. *Carex stenophylla*, *Schoenoplectus* spp.) or lack these cavities (*Juncus* spp.) (Martynov & Martynov 2007; Díaz-Paniagua et al. 2015; Lambret et al. 2015b; Matushkina et al. 2016) would be needed to test this hypothesis. Further, testing for the interaction between flooding period and type of shoot may help to identify the nature of the adults' preference.

Fourth, with respect to the type of shoot, there was little inter-shoot variance in hatching date yet considerable variance in hatching success. However, this variance was lower in *B. maritimus*. Inter-shoot variance in hatching date could be partly explained by differences between full-sib families (De Block et al. 2005; Śniegula et al. 2016). Indeed, eggs in the same shoot have a higher chance of being from the same full-sib family, although several females may oviposit in the same shoot (Lambret et al. 2015b). Several reasons may underlie the inter-shoot variance in hatching success: there may be some variation in the number of eggs laid compared to the number of incisions (Gower & Kormondy 1963; Lambret et al. 2015b) and in the degree of parasitism by Chalcidoidea wasps (Grunert 1995; Reinhard & Gerighausen 2001). Both sources of variance would not affect hatching date but could affect the hatching success values we computed. However, these biases are expected to affect all shoot types. Our results therefore show that hatching date and hatching success are more constant from one shoot of the preferred *B. maritimus* to another. In other words, laying eggs in one shoot of this oviposition plant leads the offspring to higher fitness less haphazardly.

The preference of adults for *B. maritimus* – better fitness during oviposition through higher oviposition rate, lower predation risk, etc. (Lambret et al. 2015b) – is in accordance with the hatching success of their offspring and expected chance of larvae to reach emergence. Eggs fitness is therefore likely to have selected adult behaviour by increasing their reproductive success.

#### *Implications for conservation*

A general recommendation for habitat management of this threatened species is to favour the growth of *B. maritimus* by setting a temporal flooding scheme (with a drought season lasting from late spring to autumn) and a grazing scheme limited to one season (preferably during winter) and to a low pressure (Mésleard et al. 1999). One should also avoid the invasion of non-native plant species replacing *B. maritimus* (e.g. Mattingly & Flory 2011). When combining adult higher oviposition rate in

some other plant species (Lambret et al. 2015b) and egg survival (this study), *L. macrostigma* fitness can be acceptable relative to *B. maritimus* when eggs are laid in dead plant matter of *Juncus* spp. Hence, wildlife managers are encouraged during habitat restoration programs to target those oviposition plant species by transplanting *B. maritimus* in the deeper parts of the temporary ponds and possibly *J. maritimus* and *J. acutus* on their banks. Another general recommendation for wildlife managers is to pay attention to water levels and, if able to control hydrological period, to flood temporary ponds after the drought season during autumn and not later. Given the threat of global warming and increasing duration of drought periods, this last recommendation is especially pertinent (Samways 2007; Sgolastra et al. 2011).

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## General discussion

This PhD thesis filled important gaps of knowledge on *Lestes macrostigma* biology and ecology, especially in the egg and larval stages. This species is of particular importance, first as a threatened species at regional and national scales, second as a flagship species for the conservation of temporary brackish ponds. In the first part of the General discussion, we will place this work in the general context of the research for insect conservation. In the second part, we will integrate its results, together with pieces of previous studies and general literature, in order to draw *L. macrostigma* life-history. In the third part, we will consider how to take into account these results for the conservation of the species and the management of its reproductive sites. These recommendations can also apply when using this species as a flagship to restore temporary brackish ponds, either by changing conservation management practices or by habitat creation. We will focus on the physical parameters, and especially on the hydroperiod and salinity levels, that should be set up to favour *L. macrostigma*. In this regard, we will discuss factors related to climate change that are likely to hinder future conservation efforts and suggest how to subvert them. We will also consider biological features, focusing on the restoration of aquatic plants and helophyte species that are preferred for oviposition. Further, before implementing conservation actions for one species at a large-scale, it is essential to conduct several experimental projects to test restoration methods (Schultz 2002). Hence, additional text-boxes about pilot restoration actions I conducted over the past decade in the Camargue, using *L. macrostigma* as a flagship, will resume some field evidences. Finally, we will make suggestions for upcoming research and actions, and then conclude this thesis by take-home messages.

### *Threatened insects in European conservation strategies*

If the former European Union Bird Directive targeted the conservation of avian species, the Habitats Directive and the Natura 2000 networks contribute to the conservation of 'every' species, including insects. Yet, these policies are often not sufficiently efficient for some species or group of species, which are therefore in need of additional conservation efforts. The implementation of appropriate conservation and management actions for threatened animal species requires a detailed knowledge of their biology and habitat requirements. Yet, conservation efforts and research were primarily, and

are still, overweighed toward vertebrates to the detriment of 'in'-vertebrates, i.e. those species that do not have backbones (Donaldson et al. 2017; Webster et al. 2023) – which has not much taxonomic meaning. Of the species receiving a protection status by the European Union Bird and Habitats Directives, more than 86% are vertebrates (Collen et al. 2012). This is, at least for a large part, because the empathy level of people negatively correlates with the divergence time separating us from the considered organisms (Miralles et al. 2019). In other words, the further one taxon is phylogenetically from *Homo sapiens* the less attention it receives. As so, the empathy and compassion scores of insects range as low as that of echinoderms and plants (Miralles et al. 2019). Such societal preferences pulled research activity away from less charismatic species (Troudet et al. 2017). However, threatened species are not only vertebrates, and insects are among the taxa facing the strongest decline (Dunn 2005; Sánchez-Bayo & Wyckhuys 2019). Although these neglected species recently received more attention (Collen et al. 2012), conservation research still insufficiently considers conservation priorities and threatened species especially in need of conservation efforts (Di Marco et al. 2017).

Detailed studies of insects threatened in Europe are most often lacking and very few of these species were studied extensively. For example, if general guidelines can be provided to favour *Graphoderus bilineatus* (Kolar & Boukal 2020), the rare studies concerning this globally 'Vulnerable' diving beetle often deal with distribution and habitat description (Cuppen et al. 2006; Knoblauch & Gander 2019; Turić et al. 2021). Our knowledge of the embryonic development, larval ecology and population genetic structure of the globally 'Vulnerable' Splendid cruiser *Macromia splendens* relies on three studies only (Dommanget 2001; Leipelt & Suhling 2005; Amorin et al. 2010). The Marsh fritillary *Euphydryas aurinia* was largely studied (e.g. Anthes et al. 2003, Smee et al. 2011, Casacci et al. 2015, Junker et al. 2021) but this butterfly is globally 'Least Concerned'. Contrastingly, the globally 'Vulnerable' Southern damselfly *Coenagrion mercuriale* was repeatedly studied and has benefited from conservation actions for so long (e.g. Kerry 2001; Purse et al. 2003, Rouquette & Thompson 2005, Watts et al. 2005; Keller et al. 2013) that an evidence-based conservation management handbook could be written (Dalley 2016). Noteworthy is that the great majority of the species cited above, including *L. macrostigma*, are butterflies or dragonflies, i.e. insects that are conspicuous and colourful. Hence, societal preferences also bias the identification of conservation priorities for insects. Indeed, the IUCN extinction risk of insects is so far limited to dragonflies, orthopterans, butterflies, hoverflies, bees and saproxylic beetles (Kalkman et al. 2010; Van Swaay et al. 2010; Nieto et al. 2014; Hochkirch et al. 2016; Cáliz et al. 2018; Vujić et al. 2023). There is therefore a need for more studies on other insect groups allowing the assessment of their extinction risk.

Further, there is a discrepancy between the lists of threatened species and protected species (Cardoso 2012; Hok Yau Tang & Visconti 2021). For example, there is not a single protected species

belonging to Hymenoptera and Diptera, although they represent more than 40% of European insects and that many of them are threatened (Nieto et al. 2014; Leandro et al. 2017; Vujić et al. 2023). Actually, the process of establishing the European protected insect species list also suffered several biases: Leandro et al. (2017) found that those are larger, better known, more widespread and more multi-coloured, with a higher proportion of butterflies, dragonflies and grasshoppers, and of nectarivorous and saproxylophagous species. Hence, revising the list of protected insect species therefore appears as another need, which should be made regularly and with focusing on key species for the conservation of the whole entomofauna and habitats (Leandro et al. 2017). Yet, revising dynamically this list for a better consideration of the species extinction risk may also advocate for the removal of currently protected but least concerned species. Such opening of Pandora's box may lead to conservation controversy as does the debate on bird adaptive management (Nichols et al. 2007). As many conservation actions are undertaken in favour of protected species, this discrepancy may make the conservation strategies of the European Union irrelevant (Mammides 2019). Of the ca. 800 projects funded by the LIFE Programme, only 7% targeted 'invertebrates' or plants (while 50% targeted birds), highlighting that this taxonomic bias cascades down the allocation of EU's conservation budget. Yet, the knowledge brought by Chapters 2–5 (published papers) allowed including *L. macrostigma* to the LIFE Programme 'Conservation of Pomorie Lake coastal lagoon' in Bulgaria, where the species is 'Critically Endangered' (Popov 2015). During this programme, *L. macrostigma* populations were surveyed along the Bulgarian Black Sea coast using water salinity levels, larval densities at emergence and habitat features (Chap. 3, 4 & 5). This led to the assessment of the conservation status of the species at the national scale and to recommendations in order to maintain populations and restore the species habitat where needed (Lambret et al. 2023a, 2023b).

This PhD thesis (Chap. 1–4), in addition to previous works (see General introduction, *Study species*), represents a series of studies about a threatened damselfly species from the egg to the adult stage. This integrated life cycle approach, implemented over ca. a decade, hence provides key knowledge to undertake efficient conservation actions in its favour. Such integrated life cycle approach is therefore relevant for the conservation of one species. However, habitat destruction is the main cause of species extinction (Tilman et al. 1994); and species extinction rate is the highest in insects (Dunn 2005); and the extinction risk of most insects is unknown (see above); and the number of insect experts is decreasing in Europe (Hochkirch et al. 2022). It seems therefore unlikely that this species-centred approach could be undertaken for all threatened species before they go extinct. Hence, a threatened habitat-centred approach would be more efficient for the conservation of insects, especially for restricted habitat specialists. Meanwhile, it is worth using a flagship species in order to facilitate the implementation of conservation actions when targeting one specific habitat (see General

introduction). *Lestes macrostigma* is typical of temporary brackish waters, a type of habitat that has dramatically disappeared during the last decades, and likely to shelter other specialist insect species of which extinction risk has not been assessed yet. Hence, the strategy of conserving *L. macrostigma* can be levelled up by using this charismatic insect as a flagship for the conservation and restoration of its threatened typical habitat, with most probably cascading benefits for other species. Yet, the species cannot be considered as an umbrella species (see General introduction, *Using Lestes macrostigma for the conservation of temporary brackish ponds* for definitions). If the successful establishment of *L. macrostigma* in the restored habitats would fail, these newly created pond would still contribute in increasing the number and total surface area of wetlands, hence would serve the conservation of freshwaters and their biodiversity in general. Besides aquatic insect taxa, the conservation efforts targeting *L. macrostigma* may contribute to the conservation of other threatened species such as the European 'Vulnerable' hoverfly *Lejops vittatus* (Vujić et al. 2023) which has close ecological requirements and has been observed at some *L. macrostigma* reproductive sites (É. Gaget, pers. comm.). This would make the use of *L. macrostigma* as a flagship species even more relevant.

### *Lestes macrostigma* life-history

The factors I studied and for which I concluded these are having positive, negative or no effects on the different *L. macrostigma* life-history stages are summarized at the end of this section (Tab. D.1).

#### *Egg stage*

Insects inhabiting temporary fresh waters show various adaptations to the drought period, with traits common to the most successful taxa including the possession of a diapausing or otherwise protected egg, and a highly flexible life cycle (Williams 1996). In the Camargue most of adults of *L. macrostigma* start flying (i.e. emerge) during the first fortnight of May and eggs are mainly laid during June, underlying that the egg enters a diapause after being laid and before hatching (see General introduction, *Study species*). This diapause is entered at an early developmental stage, when the egg is more resistant to the physical environment compared to later developmental stage (Chap. 1). As so, increasing salinity levels have no effect on embryonic development (Chap. 2). Further, *L. macrostigma* eggs, within their plant shoot, are able to resist to desiccation up to the next early spring (ca. 10 months after being laid, i.e. through summer higher temperatures and winter lower temperatures), yet with lower survival (Chap. 4: first experiment). Egg survival decreases when the plant shoot they were laid in is embedded in the ice (Chap. 1). Egg survival is higher and post-diapause embryonic development is faster when eggs were laid into the Sea club-rush *Bolboschoenus maritimus* regardless to other

factors (Chap. 4: second experiment). Besides, as this plant species grows in the deeper parts of brackish temporary habitats, eggs laid in this substrate have a higher chance to be flooded earlier (reduced desiccation risk) and more protected from embedment in ice (lower temperatures).

Wetting is mandatory for post-diapause embryonic development: first hatching larvae needed 20 days at 15 °C (mean, SD = 1.9, n = 20) after wetting in April although eggs had been kept outdoor, at similar temperatures for weeks (Chap. 4: first experiment). However, eggs flooded in autumn do not hatch directly, showing that diapause is mandatory as well. Williams (1996) stressed the temperature-linked development of insects inhabiting temporary waters. In average, 27 days were needed for post-diapause embryonic development after transferring flooded eggs from 3 °C to increasing temperatures (i.e. 10, 15 and 20 °C, mean = 16 °C over these 27 days; see Chap. 2). The key role of temperature is supported by the highly synchronised character of hatching within one pond, and the slight difference of mean hatching dates between ponds although most of eggs hatch during the second fortnight of March in the Camargue (Chap. 1). This is further supported by the differences in the species phenology across a latitude (and hence temperature) gradient (e.g. Benken & Raab 2008; Díaz-Paniagua et al. 2015). In the triggering of post-diapause embryonic development, besides wetting and temperature, the increase in photoperiod after winter may also control the transition from regulatory to pre-emergence development (Norling 2021). The role of photoperiod was not clearly identified in this PhD, although we found a minimal threshold of ca. 10.7 h daylight, corresponding with 20 February (Chap. 4). Taken together, these egg resistance and embryonic development characteristics fall under the plasticity of the species life cycle and its adaptation to the uncertain timing and duration of flooding conditions of Mediterranean temporary ponds, i.e. when (timing) and how long (duration) they occur.

### *Larval stage*

Larval development may start (egg hatching) when water temperature has reach a certain threshold after winter, ca. 12 °C (Chap. 4). Yet, given the complex interplay between temperature and photoperiod during post-diapause embryonic development (Norling 2021), larval development may start at lower temperature but after a longer time after diapause. Computing the difference between the EM<sub>50</sub> (see Corbet 2004) dates of hatching and emergence in one pond in Camargue, i.e. when 50% of individuals have hatched –28 March 2012 (Chap. 2)– and emerged –19 May 2009 (see General introduction, *Study species*)– respectively, the duration of *L. macrostigma* larval development would last ca. 52 days. Yet, the date of first emergence vary between years, depending on April air temperature (linear model,  $\chi^2 = 28.456$ ,  $p < 0.001$ ; Fig. D.1). The longer mean durations of larval

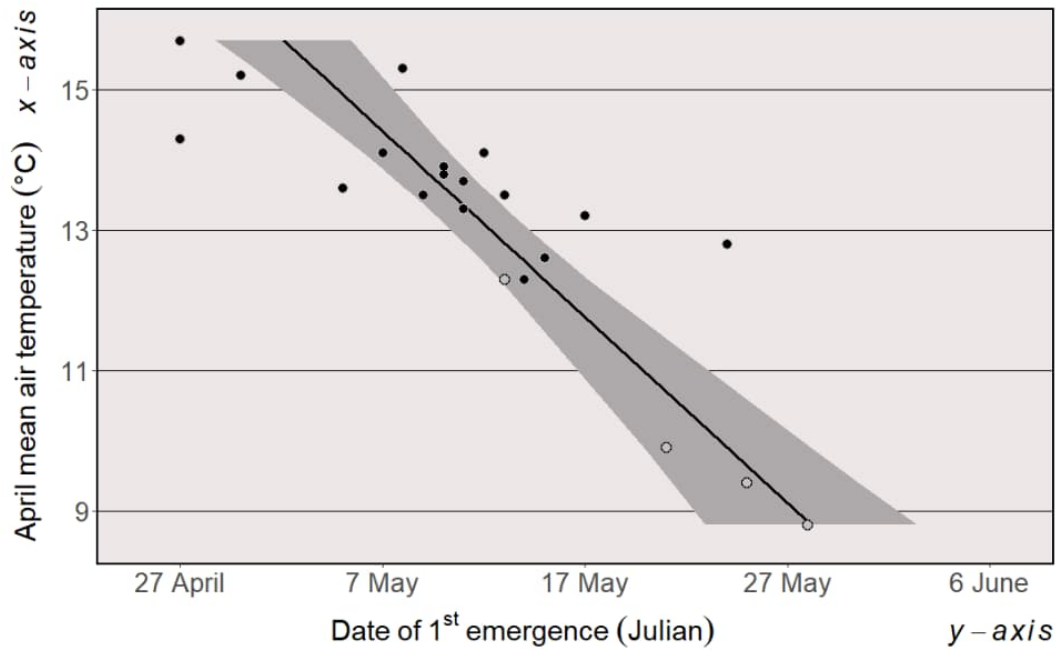


Figure D.1 – Effect of April mean air temperature on starting of *Lestes macrostigma* flying season in the Camargue (note that x- and y-axis are inverted to ease reading of dates). The black line indicates the fitted dates and the dark grey band the 95% confidence intervals estimated by the linear model. Dots indicate the observed values (grey: 1955–1958, Aguesse 1961; black: 2004–2023, unpubl. monitoring).

development during our two laboratory experiments (65–74 days; Chap. 2) were certainly biased by the fixed incubating conditions, and especially the photoperiod (13|11 Light|Dark and 12|12 L|D in experiment 1 and 2, respectively). Indeed, increasing daylight duration indicates time constraint and accelerates larval development (De Block & Stoks 2004). Rearing the species under natural photoperiod, Schiel & Buchwald (2015a) observed a mean larval development time of 51 days, which is congruent with our assessment from field data.

The date of emergence may also vary with pond depth. Indeed, shallower waters show higher temperature fluctuations (Martin 1972; see also Chap. 1) and this constraints larvae to grow at a lower rate (Verheyen & Stoks 2019). One should therefore expect larvae to emerge latter from shallower ponds. Instead, we observed that adults emerge slightly earlier from shallower waters (unpubl. monitoring) and at a smaller size (Chap. 3). We hypothesized that this was because shallower waters have higher mean temperature, which accelerates the larval pace-of-life, with increased growth rate and decreased development duration. Yet, regardless to daily fluctuations, mean temperatures may be similar in deeper and shallower waters (Chap. 1). Hence, an alternative explanation would be that larvae growing in shallower waters with higher temperature fluctuations may do so at a lower rate and emerge at a smaller size because of time constraint indicated by the daylight duration (De Block &



Stoks 2004). By contrast, larvae growing in deeper waters may do so at a higher growth rate and emerge at a larger size (alternative hypothesis than that we made in Chap. 3). In any case, given that clutch size and longevity show a positive relationship with body size (Sokolovska et al. 2000), deeper ponds may positively affect adult lifespan and fecundity.

Insects of temporary habitat face the challenge to achieve their larval development before the habitat dries out and to do so, they usually show larval peculiar life-history traits such as a rapid growth and development (Williams 1987; Strachan et al. 2015). *Lestes macrostigma* has the fastest larval development and the highest growth rate among the species of its genus (Schiel & Buchwald 2015a, 2016a). This underlines the adaptation of the species to the vernal character of its typical aquatic habitat. This typical habitat is also most often brackish, and salinity levels increasing with evaporation could serve as a cue of a drying temporary habitat. However, *L. macrostigma* does not accelerate its life-history under salinity stress (Chap. 2). We found that *L. macrostigma* is a saline generalist with a broad fundamental niche, a pattern found in many saline insects (Arribas et al. 2019). Indeed, its larvae are able to grow from hatching to emergence in water salted from 0.5 to 8 g/L, but all those doing so in 16 g/L water die before they reach the adult stage (Chap. 2). Yet, larvae can emerge from water salted up to 28 g/L, although with low success, given that such salinity level is experienced during only one bout of larval development (Lambret et al. 2009; Chap. 3). Further, larval survival increases with salinity level increasing from 0.5 to 8 g/L, and then declines at 16 g/L. However, it performs better at lower salinity levels, as increasing salinity levels have sublethal negative effects such as reduced growth rate and increased development time. Negative effects bridge to the adult stage with smaller size at emergence and lower net energy budget with increasing salinity levels experienced during the larval stage, these effects being often expected to decrease lifetime mating success. Yet, in species having larvae growing in unpredictable habitat and hence with unpredictable survivorship, adult mating success may be uncoupled from their realized fitness. For example, Fincke & Hadrys (2001) showed in the tree hole damselfly *Megaloprepus coerulatus* that female clutch size and male mating success were correlated with body size but poorly predicted their offspring survival, contrarily to the span over which their eggs hatched. Hence, mating success of *L. macrostigma* could overall be higher in ponds with moderate salinity levels though a higher larval survival, in absolute terms and because of lower predator abundance (Gazaix et al. 2023), and despite the negative sublethal effects increasing salinity levels have.

Fundamental ecological requirements are related to the physio-chemical environment and are then restricted by biotic interactions, leading to the realized niche (Soberón & Peterson 2005). The relative abundance of larger aeshnid larvae has a negative effect on *L. macrostigma* larval abundance (Chap. 3). We hypothesized that this is due to higher predation level by aeshnid larvae, and therefore

a lower survival of *L. macrostigma* larvae. The latter would be exposed to a higher predation risk because they may maintain a foraging activity in the presence of predators to achieve their growth and development as fast as possible, and thereby increase their chance to emerge before the aquatic habitat dries out. We also found that the later the flooding date of the temporary habitat after the summer drought, the smaller the aeshnid and the higher *L. macrostigma* larval density. Further, the abundance of aeshnid larvae decreases with increasing salinity levels (Gazaix et al. 2023). Hence, we expect that lower predation level mediates the shift of the species toward temporary brackish waters, its realized niche. Biotic interactions may also consist in the relations between larvae and aquatic plants, as these are well known drivers of odonates larval communities, i.e. species richness and abundance (e.g. Huikkonen et al. 2020; Perron et al. 2021). *Lestes macrostigma* larvae are plant-dwellers (see General introduction, *Study species*), suggesting that hydrophytes is an important microhabitat feature for the species. However, we did not identify hydrophyte abundance as a strong driver of *L. macrostigma* larval densities (Chap. 3), meaning that the effects of other habitat features, either biotic or abiotic, are greater than that of aquatic plants.

#### *Oviposition site selection (adult stage)*

The distribution of aquatic insects varies according to different spatial scales, reflecting nested habitats with one macrohabitat including several mesohabitats, which may themselves include several microhabitats (e.g. Sheldon & Walker 1998; Garcia-Roger et al. 2013). For instance, Mediterranean intermittent streams (macrohabitat) consist in successive riffles and pools (mesohabitats), the bottom of which may vary in grain size – e.g. clay or gravel – and organic cover – e.g. algae or submerged macrophytes – (microhabitats). This means that adults may select their habitat, and therefore their oviposition site, from the macrohabitat (including landscape) to the microhabitat scales, possibly in interaction (e.g. Hoffmann & Resh 2003; Binckley & Resetarits 2007; Kadoya et al. 2008). Insect offspring (either eggs or larvae) may gain fitness benefits from their parents' reproductive behaviour, especially during oviposition site selection, but this expected relationship is often decoupled or may vary according to the fitness component that is considered (e.g. Simmons 1987; Sadeghi & Gilbert 1999; Rivero 2000; Noriyuki 2015). In *L. macrostigma*, we found evidences of adult preferences benefiting their offspring fitness at the meso- and microhabitat scales.

First, at the mesohabitat scale, *L. macrostigma* larval density is higher in smaller ponds, suggesting that adults prefer to lay eggs in these compared to larger ponds (Chap. 3). Dragonflies are able to select ponds regarding their size (Kadoya et al. 2004). Further, insect females may select their oviposition site especially to reduce the predation risk for their offspring (e.g. Blaustein 1999; Brodin

et al. 2006). Given the susceptibility of *L. macrostigma* to aeshnid predators, we hypothesized that pond size would serve as a cue for predation risk. Hence, adult preferences at the mesohabitat scale would increase offspring survival through reduce predation risk. However, higher densities of conspecifics may be detrimental to other fitness components, including at the adult stage. For example, Cardoso-Leite et al. (2014) showed that *Lestes bipupillatus* larvae emerged earlier when experiencing higher conspecific density during their last larval stage, suggesting that they accelerated their pace-of-life and emerged at smaller size. Contrarily, De Block & Stoks (2005b) found that *L. viridis* larvae growing in drying pond conditions (i.e. decreasing water levels), with higher conspecific densities and lower food levels and hence higher competition, had a slower growth and emerged later, yet also resulting in smaller adults. Smaller adult body size is known to have cascading negative effects on their longevity and clutch size (Sokolovska et al. 2000). Yet, Thompson et al. (2011) showed in *Coenagrion puella* that the number of offspring surviving to maturity was explained by adult longevity but only partly, in addition to other factors such as environmental conditions or population size. Again, adult mating success can be uncoupled from their realized fitness (Fincke & Hadrys 2001) and larval survival is a major factor driving the damselfly population dynamics (McPeck 2008). Hence, although higher larval densities may have some negative consequences on offspring fitness, the preference *L. macrostigma* adults have for smaller ponds (mesohabitat) during oviposition likely leads to larger population the next year through overall higher larval survival.

Second, at the microhabitat scale, the endophytic egg-laying females of *L. macrostigma*, show a clear preference for the Sea club-rush *B. maritimus* during the two first steps of the oviposition site selection process, which are (i) the initial choice and (ii) the insertion site choice (Lambret et al. 2015a, 2015b; see General introduction, *Study species*). Further, eggs (i.e. embryos) laid into *B. maritimus* have a higher survival, a faster development, a reduced desiccation risk and a likely higher protection from lower temperatures (Chap. 2 & 4). Yet, in ponds where *B. maritimus* is poorly available or even completely lacking, *L. macrostigma* females can use several other plant species to lay their eggs. Successful hatching from these alternative oviposition substrate lead to the sustainability of local population (Lambret et al. 2009). This shows a certain opportunism of the species during oviposition site selection, despite its preference for *B. maritimus*, and further illustrate its plasticity regarding the unpredictability of its typical habitat.

Table D.1 – Summary of the effects of the factors tested in this PhD on the different *L. macrostigma* life-history stages. Green and orange colours indicate positive and negative effects, respectively. Grey shading indicates that the effect was not tested.

Chapter	Factor	Egg	Larva	Adult
1	Embedment in ice	decreased survival	reduced population size	reduced population size
2	Increasing salinity 0.5–9 g/L	none	increased survival	increased population size
		none	increased development time, decreased growth rate	decreased net energy budget, decreased size at emergence
	Increasing salinity 8–16 g/L		decreased survival	decreased population size
3	Smaller ponds		higher density	
	Deeper ponds			increased size at emergence
	Later flooding date	decreased survival	lower predation (aeshnid limited in size)	
4	Laying eggs in <i>Bolboschoenus maritimus</i>	increased survival, faster development		faster egg deposition rate (Lambret et al. 2015b)

### *Conservation practices to favour Lestes macrostigma and to restore temporary brackish ponds*

Conservation practices should be based on reviewed available information instead of common sense, personal experiences or expert/managers advices (Sutherland et al. 2004). Following this evidence-based conservation approach, pond management and restoration should be based on science (Sayer & Greaves 2020) and it is essential to transfer scientific knowledge to practitioners for undertaking efficient actions in situ (e.g. Oertli et al. 2009; Segan et al. 2011). Hence, the knowledge brought by this PhD about *L. macrostigma* ecology at the egg and the larval stages needs to be translated into conservation practices to favour the species. We will consider especially the hydroperiod, the salinity levels and the vegetation. Habitat restoration sensu lato (see General introduction) may consist in various actions, from the mere change in management practices (e.g. irrigation schedule, grazing pressure) to the creation of new habitats (e.g. pond excavation). As so, the following recommendations in favour of *L. macrostigma* can apply to management plan or guide the restoration of MTBPs when using the species as flagship.

*Hydrology and pond design*

Hydroregime and salinity drive plant and animal – including insect – communities (see General introduction). These factors also shape *L. macrostigma* population size and therefore dynamics (see Chap. 1–4). Despite the numerous factors influencing the success, or failure, of wetland restoration actions (e.g. surrounding landscape, soil properties, topography, seed bank, invasive species; see Zedler 2000), one first step consists in the restoration or settlement of a defined hydroperiod (e.g. Gilpin & Vowell 2006; De Steven et al. 2010; Toth 2010). Flooding of temporary ponds in late autumn or early winter, rather than late summer or early autumn, increases *L. macrostigma* larval densities in the next spring (Chap. 3). This seems mediated by the aeshnid dragonfly larvae, as the size and relative abundance of these predators were lower in later flooded ponds. In the Camargue, flooding temporary ponds in July or August is a typical practice set up by gaming managers. They do so in order to favour early growth of aquatic plants and thereby attract the waterfowl when the hunting season opens, at the end of August. This water management practice is therefore unfavourable to *L. macrostigma*, but also to other Mediterranean species (Chap. 3). Further, since *L. macrostigma* larvae hatch from mid-March in southern France (Chap. 1), one could target a beginning of the flooding period in early-March. Yet, flooding as late as in February dramatically decreases egg survival compared to flooding in November (Chap. 4). Taken together, these results suggest that the optimal timing for flooding *L. macrostigma* temporary brackish ponds is November. Further, given that late larvae may hatch in mid-April (Chap. 1) and given the time they need to reach the adult stage (see *Larval development*), last larvae may emerge in early-June, and temporary brackish pond should not dry earlier. This is supported by Berquier et al. (2022) who found that the abundance of emerging adults increases with later drying of the pond.

In the Camargue, the March water levels of ponds where *L. macrostigma* reproduces range from 16 to 75 cm with a mean of 38 cm (Chap. 3). Among this range, the species prefer deeper ponds compared to shallow waters for several reasons. First, adults tend to be bigger when they emerge from ponds with higher water levels (Chap. 3). Second, embedment of overwintering eggs in ice increases their mortality (Chap. 4). In case of a cold wave in winter that would freeze the pond surface, eggs at the bottom of deeper ponds would have a lower risk of mortality compared to those in shallow waters. Third, considering the target hydroperiod, deeper ponds are more resilient to hydroclimatic fluctuations and more likely to maintain surface water during high evapotranspiration in April–May. Hence, we suggest maintaining a water level of at least 30 cm until the beginning of the emergence period (Chap. 3). Further, new ponds to be created should be designed with a maximal water depth of ca. 80 cm below the surrounding ground surface, i.e. above the pond bottom. The size of the pond is another important feature to consider. Pond size influences many biological communities such as

those of crustaceans (Ripley & Simovich 2009), insects (Oertli et al. 2002), amphibians (Werner et al. 2007) and birds (Sebastián-González & Green 2014). Most of odonates seem to prefer larger ponds as these show a higher species richness compared to smaller ponds (Oertli et al. 2002; Kadoya et al. 2004). However, the total abundance of *L. macrostigma* larvae is higher in smaller ponds, likely because these are especially selected by adults during oviposition as such ponds may indicate a lower abundance of predators (Chap. 3). Hence, we suggest to create ponds ranging from ca. 200 m<sup>2</sup> (0.02 ha) to 1,000 m<sup>2</sup> (0.1 ha). Restoring a network of smaller ponds of varying size and depth leads to a higher regional biodiversity than restoring one larger pond (Oertli et al. 2002; Sebastián-González & Green 2014). Contrarily to polarised light (e.g. Bernáth et al. 2002) and vegetation structure (e.g. Buchwald 1992), pond shape is not reported to drive habitat selection by odonates and seems therefore of minor importance besides aesthetics. By contrast, the slope of the banks and the location of the pond deepest part (i.e. pond profile) may be important features. Indeed, in case of low water level at the time of emergence, larvae may use a steep bank as alternative emergence support (see Text-box 1a). We therefore recommend designing pond profile with one steep bank bordering the deepest area of the pond.

In the case of a natural hydro-functioning of the pond (i.e. without the possibility of managing the water levels through controlled flooding), its hydroperiod will depend, besides on its depth, on how much water the pond obtains especially from rainfall (see General Introduction, Fig. I.1), and therefore on the dimensions of its watershed and on the precipitation pattern. Hence, the size of the watershed may be adjusted to reach the target hydroperiod through removing, rearranging, or plugging canals, ditches, culverts or dikes (e.g. De Steven et al. 2010; Toth 2010). The hydrology of the wetland will also be influenced by its geomorphology (Gilpin & Vowell 2006). Hence, in the case of new pond excavation, it is essential to control beforehand the clay content of soil, i.e. the presence of low permeability soil layer, in order to avoid pond water to infiltrate into the soil. This may help to refine the pond profile (i.e. its maximal depth) but also its location within the watershed. Therefore, when designing a new pond of which water levels will only depend on rainfall, it is essential to model the future hydrology using topographic, edaphic and hydroclimatic data (Price et al. 2010; Text-box 1a). However, modelling robustly the future filling of the pond by rainfall may be tricky as there is growing evidence that climate change disturbs the precipitation regime in the Mediterranean area, yet with variable strength at smaller scales within this area (Zittis 2018). Especially in southern France, while

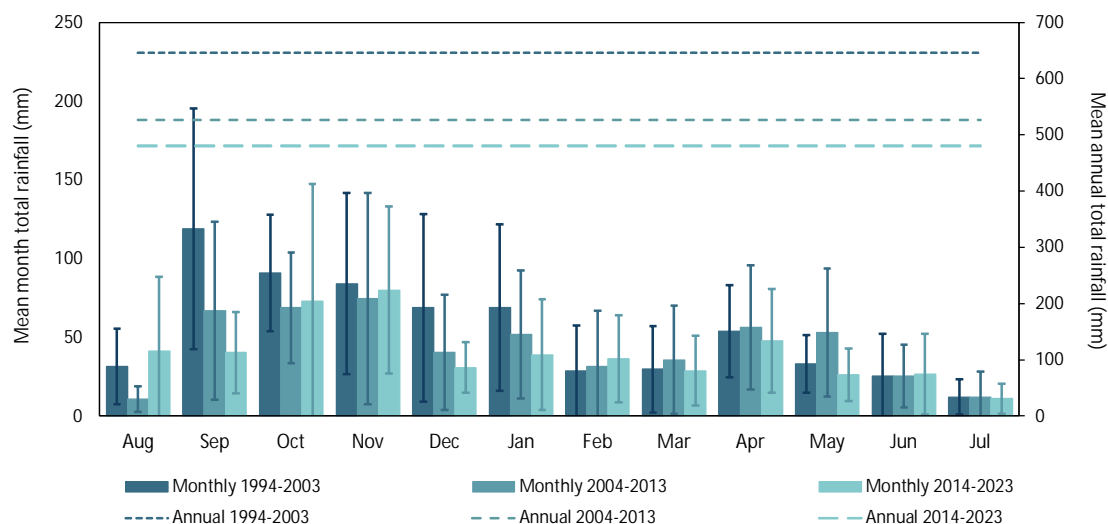


Figure D.2 – Mean total rainfall at the Tour du Valat during the last three decades (bars indicate standard deviation of the monthly means; data: Météo France).

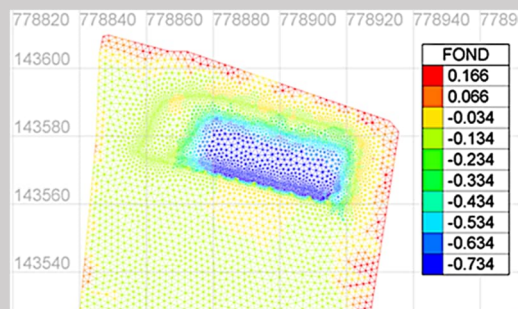
mean total precipitations are expected to decrease in the future, daily rainfall extremes should increase (Zittis et al. 2021). Further, increasing temperatures will contribute to higher evapotranspiration levels and lower water availability (Dezsi et al. 2018). In the Camargue, over the last 30 years, total rainfall decreased by ca. 26%: from  $645 \pm 182$  mm (mean  $\pm$  SD) during the period 1994–2003, to  $526 \pm 147$  mm in 2004–2013 and then to  $481 \pm 205$  mm in 2014–2023, with monthly totals during this later period significantly lower than in 1994–2003 (GLMM,  $z = -2.372$ ,  $p = 0.018$ ; data: Météo France, meteorological station n°13004003 of 'Arles-Valat',  $43^{\circ}30'36''\text{N}$  |  $4^{\circ}41'38''\text{E}$ ; Fig. D.2). This decrease especially occurred in winter (December and January), when temporary ponds should be flooded to limit *L. macrostigma* eggs desiccation risk (Chap. 4). Also, over the period 1992–2023 in the Camargue, April mean air temperature has significantly increased (linear model,  $t = 2.106$ ,  $p = 0.044$ , data: Météo France, op. cit.; see also Fig. D.1), resulting in higher evapotranspiration during *L. macrostigma* larval development (Chap. 1). Consequently, during these last years, many temporary ponds that were only flooded by the rain dried out before *L. macrostigma* larvae emerged, and the species could successfully reproduce only in pond and marshes of which water levels are controlled by conservation managers (unpubl. monitoring; see also Text-box 1b). Given that *L. macrostigma* egg-bank is not perennial (Chap. 4) it is important for the species conservation that at least some ponds are flooded enough for a successful reproduction. The future habitat restoration projects should therefore include some refuge pond with an irrigation network allowing the supply of water to guarantee the target hydroperiod (Text-box 1b).

## Text-box 1a – Restoring hydroperiod: designing ponds with endoreic functioning

In the framework of the National Strategy for Biodiversity, a project entitled ‘Restoration of Mediterranean temporary ponds’ aimed at creating in 2013-14 several endoreic ponds (i.e. only flooded by rainfall) on previously man-levelled parcels (Lambret 2017). Ponds shape (incl. size) and profile (i.e. depth and bank slopes) were designed using advices from different experts (conservation managers, hydrologists, botanists, entomologists). Ponds differ in shape but all feature a maximal depth of ca. 80 cm and at least one low sloping bank to allow the establishment of several plant communities. Some also feature stiff banks bordering especially the deepest part of the pond (opposite picture).



Hydrologic modelling was made after excavation, to correct pond design if necessary. Topographic data of the ponds and their watersheds were obtained using a tacheometer. A two-dimensional (i.e. surface) digital elevation model with triangular mesh was built in order to figure water levels (see figure below: pond watershed topography, with elevation [i.e. altitude] given relative to a point located at the centre of the watershed, and geographic coordinates [Lambert 93 metres]; realisation: J. Formento | Tour du Valat). The mean hydric deficit was defined as the difference



between mean cumulated rainfall and mean cumulated evaporation, computed with local meteorological data using year data from 1<sup>st</sup> August to 31<sup>st</sup> July over the period 2005–14. Then, flooding durations were simulated using Telemac 2D software during a year with average rainfall and evapotranspiration. This revealed that ponds would most likely show the target hydroperiod, even during ‘dry’ years.

Since then, *L. macrostigma* reproduces successfully, yet not every year for unclear reasons, in five of the seven created ponds that are monitored. Ponds without emerging vegetation and featuring only low sloping banks lack emergence supports, so that during years with excessive water deficit, larvae willing to emerge risk staying in the aquatic environment and die. Conversely, in ponds with stiff banks (opposite left picture), larvae may climb on one of these bordering the deepest part of the pond where water remains to reach vegetation and emerge, as did the larva that left its exuvia on a samphire shoot (opposite right picture). (Pictures © P. Lambret.)



Supplying freshwater may also help maintaining salinity levels within the optimal range for *L. macrostigma* larval development by counteracting the concentration of salt due to increasing evapotranspiration during spring, especially in the context of climate change and surface water salinization (Aydin et al. 2019). Indeed, although *L. macrostigma* larvae prefer brackish waters,



## Text-box 1b – Restoring hydroperiod: water management using an irrigation network

During the dragonfly survey of the ‘green agri-environmental belt’ of the Marseille-Fos Euro-Mediterranean Port (Fig. 3.1) in 2014–16, *L. macrostigma* was found to reproduce successfully in some ponds, but not during ‘dry’ years (Lambret 2016b). In 2019–20, the environment department of this port, that is in charge of the wild-life management of the ‘green belt’, set up a restoration project as an offsetting measure of the destruction of another natural wetland. This project consisted in the settlement of an electric pumping station and the built up of an irrigation network (opposite picture, © P. Lambret) allowing drawing water from a canal and the flooding of these ponds. In May 2022, when most of the endoreic temporary ponds in the Camargue were dry already, *L. macrostigma* emerged from these managed ponds, thanks to the artificial pumping to maintain their water levels.



Through another project, we aimed at testing in situ the results about the positive effect of latter flooding date on *L. macrostigma* larval densities (Chap. 4), and therefore its reproductive success. We also aimed at testing the effect of latter flooding date on plant and macrozoobenthic communities, in order to assess the relevancy of using *L. macrostigma* as a flagship for the conservation of temporary brackish pond biodiversity. To do so, nine ponds were created in 2020 on ancient salt pans that were abandoned since the construction of the Marseille-Fos port in the 1970s (Lambret et al. 2021a). An irrigation network was built in order to control their water levels. These ponds are similar in shape, size, depth, profile and orientation, but differ in flooding type (i.e. hydroperiod timing and duration): three are flooded in early August, three in early October and three in mid-November; water levels are maintained until the end of May and then ponds dry naturally. Water salinity levels are lower in the early-flooded ponds and higher in the later-flooded ponds (Gazaix et al. 2023). Yet, salinity levels also differ between ponds of the same flooding type. Soil and ground water salinity levels where ponds were excavated were not assessed before pond creation and this important issue needs further research in order to predict pond salinity levels. However, *L. macrostigma* reproduced successfully in the later-flooded ponds (opposite picture, © P. Lambret), to the opposite of early-flooded ponds where *A. parthenope* is abundant. Further, if plants are more abundant in early-flooded ponds, some species



of conservation interest and typical of temporary lagoons only grow in the later-flooded ponds. The macrozoobenthic communities already follow different ecological trajectories regarding pond flooding type. Yet, these trajectories are only initiated and functioning of created wetlands may take decades to get close to reference ecosystems (Moreno-Mateos et al. 2015b). More monitoring is required to further assess the contribution of using *L. macrostigma* as flagship in conserving insect biodiversity.

increasing water salinity levels have negative effects on their development, these carrying over to the adult stage, and even on their survival above a certain level (Chap. 2). Yet, feeding temporary ponds through irrigation channels may enhance eutrophication and favour the introduction of pesticide residuals and species (e.g. fish, crayfish) possibly harmful to native biodiversity (Brönmark & Hansson

2002; Zacharias et al. 2007; Ito et al. 2020). Further, the use of an irrigation network may not be always possible, because of the remote location of the restored pond for example. This would advocate for designing new ponds and their watershed with a 'self-functioning' hydrology (i.e. endoreic functioning; see Text-box 1a) through the integration of hydroclimatic data and their natural fluctuations. In such case, soil salinity would be an additional variable to include in the modelling of the future hydrological functioning of the restored pond. Despite the influence of soil salinity on water salinity in the frame of restoration projects, detailed information on the relationship between these two variables are lacking (e.g. Cui et al. 2009; Takekawa et al. 2015). In coastal environment, soil salinity depends on complex interactions between e.g. vertical and horizontal water flows, soil porosity, nature of deposits (lagoonal-fluvial vs marine) and evaporation (Torres-Rondon et al. 2013). A rough assessment of data to include in the model predicting the future pond salinity, and possibly refine its design before excavation, could be made by measuring soil salinity along a depth profile. Yet, a time-lasting hydrogeological study, using piezometers to record groundwater levels and salinity, would provide more robust data in this regard (Akter et al. 2021).

To improve the conservation status of *L. macrostigma* or in the case of its use as a flagship for the restoration of MTBPs, the species ecological requirements in terms of hydroperiod timing (when it occurs) and duration (for how long) and salinity range should therefore guide water management, including dimensioning of the watershed. They may also guide the design of created ponds though their size, depth and profile. Yet, these guidelines are likely to influence pond vegetation. Indeed, the distribution of plant species along water-depth gradient of temporary wetlands depend on flooding depth, duration and fluctuations (Casanova & Brock 2000; Seabloom et al. 2001). In temporary ponds, these features will depend on, besides water inflow, topographic/depth profile. Bank slope and maximal depth drive the establishment of vegetation, with steeper banks hampering the establishment of different vegetation structures, with potentially cascading effects on invertebrate diversity (Schmitt et al. 2018; Kolar et al. 2021). Conversely, low sloping banks allow the establishment of several plant communities each corresponding to a specific hydrology. Although neither the hydrophyte abundance nor the availability of oviposition plants drive *L. macrostigma* larval densities (Chap. 3), the preferred oviposition substrate, i.e. living shoots of the Sea club-rush *B. maritimus*, maximizes egg survival and therefore increases hatching success (Chap. 4). Hence, besides the design of low slope banks, we will now consider how this preference may drive the restoration of MTBPs vegetation when using *L. macrostigma* as a flagship.

*Aquatic plant and helophytes*

Vegetation features such as diversity and patterns are key to the conservation of insects (Panzer & Schwartz 1998; Burgio et al. 2015). Especially, suitable plant species, together with their vertical and horizontal structure, are important for the colonisation of not only phytophagous insects, but also those predatory insects associated with particular plants (Mortimer et al. 1998). Odonates are known for long time to use immersed and emerged vegetation features during oviposition site selection because these indicate suitable habitat for their larvae, or conditions for egg development especially in species laying eggs endophytically (e.g. Buchwald 1992; Ward & Mill 2005; Lambret et al. 2015a, 2015b; Brito et al. 2020; see also Chap. 4). For example, fens with extended patches of floating *Stratiotes aloides* is a key for the conservation of *Aeshna viridis*, a dragonfly needing this plant species for oviposition (Lamers et al. 2002). Khelifa & Mellal (2016) showed that restoring *Potamogeton nodosus*, the preferred oviposition plant of the endangered *Calopteryx exul*, led to an increase in the damselfly population size and philopatry level. Yet, the colonisation of created ponds by the vegetation takes much more time than colonisation by active insect dispersers (Moreno-Mateos et al. 2015b; Coccia et al. 2016). Further, successful restoration of a target hydroperiod and wetland depth may not lead to the development of the target plant communities (Toth 2010). Williams et al. (2008) surveyed 40 excavated ponds in the U.K. and found that natural colonisation by aquatic plants was still poor seven years after pond creation. Sebastián-González & Green (2014) reported from Doñana that the establishment of *Bolboschoenus maritimus* (i.e. the preferred plant of *L. macrostigma* during oviposition) and *Schoenoplectus litoralis* was almost inexistent four years after pond creation, although these helophyte species were dominant in the adjacent natural marshes, highlighting their dispersal limits. Also, helophyte species are much more fragile when they are at a stage of establishment (i.e. germination, seedling, plantlets) compared to developed individuals, notably regarding complete flooding or drought (P. Grillas pers. comm.).

In fact, the establishment or the change of plant communities after setting management actions mostly results from the germination of the existing seed bank and the seed rain (e.g. De Steven et al. 2010; Sebastián-González & Green 2014). These seeds are mostly present within the upper 5 cm of the soil, whatever the type of habitat (e.g. forest: Bonis & Lepart 1994; grassland: Muller et al. 2014; temporary marshes: Godefroid et al. 2006). Hence, after creating a pond by soil excavation, the seed bank present at the bottom of this pond is far less diverse and abundant compared to that of natural ponds (Aponte et al. 2010). ‘Self-design’ projects only rely on the natural establishment of plants and, with this ‘passive’ approach of vegetation restoration, the dispersal and colonisation abilities of plant species therefore appear as a limiting factor (Mitsch et al. 2012). Alternatively, practitioners may choose an ‘active’ approach to restore vegetation. Active restoration of vegetation, or revegetation,

consists in accelerating the establishment of one plant community or forcing ecological trajectory to a certain plant community. This can be achieved through sowing seeds, transferring soil containing seeds, or planting rhizome, seedlings or cuttings, and may include actions to avoid the establishment of invasive species (e.g. Ruwanza et al. 2013; Muller et al. 2014; González et al. 2015; Hess et al. 2019; Choi et al. 2021). Noteworthy is that the dichotomy – and controversy – between passive and active approaches seems outdated because restoration techniques rather segregate along a continuum (Prach et al. 2020; Chazdon et al. 2021).

The restoration success of salt marsh vegetation may depend on planting techniques. Rabinowitz et al. (2023) compared plugging potted seeds, transplanting existing field clumps, sowing and burying wrack (i.e. dead vegetative material), and obtained higher success with plugs and transplants. In *J. maritimus* – the second preferred oviposition plant for *L. macrostigma* (Chap. 4) –, field collected transplants may have higher survival and biomass than plugs from commercial nursery (Thomsen et al. 2005). Yet, local environmental factors may influence restoration success to a greater extent than planting techniques (Rabinowitz et al. 2023). For example, increasing salinity has a negative effect on the germination of *J. acutus* (Greenwood & MacFarlane 2006). Few experiments aimed at restoring *B. maritimus* covers actively. Maynard (2014) harvested *B. maritimus* seeds close to maturity and sowed them in autumn (unprocessed) and spring (after storage in wet/cool conditions) at densities of 100 and 200 seeds/m<sup>2</sup>. But seed germination did not occur in any of the four treatments because seeds were not viable. In fact, stratification of *B. maritimus* seeds by storage in wet/cold (instead of wet/cool) conditions improves germination success (Clevering 1995). In the frame of a wetland restoration project in the Camargue, Willm et al. (2011) sampled *B. maritimus* seeds in late summer and sowed them the following spring after storage in dry/outdoor conditions during winter. This method led to the successful establishment of the species. Maynard (2014) obtained a rapid establishment of the species as well by transplanting shoots with developing bud, rhizome and associated roots. Further, Charpentier et al. (1998) compared the sprouting rate and the extent of colonisation in intact ramets (i.e. series of tubers interconnected by their rhizome) vs severed ramets (i.e. isolated single tubers). They observed that intact ramets colonised their surrounding area to further distance compared to severed tubers, while the last produced higher numbers of above-ground shoots (which are used by *L. macrostigma* during oviposition). Hence, transplanting both intact ramets of few tubers and severed tubers should therefore enhance the colonisation success of restored ponds by *B. maritimus* and rapidly offer oviposition substrate to *L. macrostigma*. This method appears more costly and time consuming than sowing or passive restoration, especially if organized at a large scale (O'Connell et al. 2013). Moreover, cost-effectiveness is a key feature to take into account

## Text-box 2 – Restoring vegetation: passive vs active approach

After the excavation of the endoreic Mediterranean ponds of the National Strategy for Biodiversity project (see Text-box 1a), the vegetation and the fauna were let to colonise the ponds naturally. The establishment speed of aquatic plants varied across ponds, ranging from inexistent eight years after pond excavation to advanced four years after, with intermediate situations. Further, a decade now after their creation, the establishment of helophytes (used for *L. macrostigma* adults during oviposition and by its larvae for emergence) ranges from inexistent to very poor, even in ponds where aquatic plants are abundant (opposite picture). At most, few shoots of *Bolboschoenus maritimus* grow nearby the surrounding *Juncus maritimus* patches that were present before excavation.



In the framework of the Marseille-Fos port project (see Text-box 1b), the vegetation of the nine created ponds was restored actively by transferring seeds of aquatic plant and helophyte species (after Muller et al. 2013). To do so, ca. 100 kg of soil were collected over 20 plots of 50 × 50 × 5 cm in each of four different temporary ponds (bottom left picture), the collects were mixed and spread homogeneously over the ponds just after their first flooding following excavation (bottom centre picture, © P. Lambret). The establishment of aquatic plants started after few weeks or months. Yet, that of helophytes requires more time, although faster than in the case of 'passive' restoration (bottom right picture). (Pictures © P. Lambret.)



in the design of restoration project (e.g. Kimball et al. 2015). However, to passive restoration may be related hidden costs such as a perception of failure if the ecosystem recovery takes too long, with the risk of abandoning the project prematurely (Zahawi et al. 2014). If vegetation establishment takes long, initial passive restoration may be followed by active restoration in order to increase the project success, which is more costly than direct active restoration (Dorrough et al. 2008). In the case of the conservation of *L. macrostigma*, this advocates for assisting colonisation of created ponds by key plant species in order to accelerate and maximise restoration success. Further, to maintain *B. maritimus* covers (either existing or restored), a grazing scheme may be adapted to the appropriate hydroperiod (see Chap.5: *Implications for conservation*). Indeed, dense *Phragmites australis* reed may take over

*B. maritimus* in absence of grazing (Mesléard et al. 1995), which is unfavourable to *L. macrostigma* dispersal (Lambret, pers. obs.). By contrast, the abundance of *B. maritimus* may severely decrease in case of overgrazing, as this species is highly palatable to cattle (Mesléard et al. 1995, 1999).

### *Suggestions for future research and actions*

This thesis provides a thorough background knowledge about *Lestes macrostigma* biology and ecology. It helps understanding the species habitat preference and, especially, its great inter-annual variations in adult abundance and in spatial occurrence, which was so far somewhat enigmatic. However, some gaps of knowledge remain and several issues should be addressed in the future to improve the conservation actions taken in favour of *L. macrostigma*:

- Chapters 2 and 4 suggest that the shift of the species' ecological niche toward brackish and temporary waters is related to a higher sensitivity to predation, possibly in relation to the fast pace-of-life of its larvae as they have to complete their life cycle very rapidly. Yet, this remains to be confirmed in controlled conditions, for instance through an experiment combining salinity levels, predator presence, and competition with a sister species (e.g. *L. sponsa*). Further, using gradually increasing salinity levels (rather than fixed; see Chap. 2) should provide a more accurate assessment of the effect of salinity as in natural ponds salinity increases with evaporation, and therefore gradually increases with time. More generally, upcoming field research should be combined with further experimental research (i.e. in controlled conditions) in order to determine causal relationships (Lamers et al., 2015).

- Undertaking efficient actions in favour of *L. macrostigma* is a priority. The species can be used as a flagship to restore or create temporary brackish wetlands, another conservation priority. Project funders usually expect rapid results through short programmes. It may therefore sound 'unproductive' if *L. macrostigma* does not colonise the restored/created habitat, although the re-creation of wetlands would benefit anyway to some freshwater biodiversity. Invertebrate colonisation and population recovery success and speed primarily depend on distance to the source population and on species dispersal ability, although other factors such as population dynamics or physical barriers may be at play (e.g. Solbreck 1991; Gee et al. 1997; Blakely et al. 2006; Watts & Didham 2006). Colonisation of restored or created ponds by odonates is fast (Minot et al. 2021). Yet, there is a lack of specific studies about *L. macrostigma* dispersal behaviour and abilities, and possible metapopulation functioning, although some field observations suggest that adults may disperse up to dozens or sometimes hundreds of kilometres, as a probable strategy to colonise new sites (see General introduction, *Study species*). Nevertheless, the probability of reaching a site decreases rapidly with the distance to the

source population. Further, given the patchy distribution of *L. macrostigma*, habitat restoration actions aiming at increasing its area of occupation may be launched far from the closest source population; likely too far for natural colonisation. The creation of stepping stones, especially in fragmented landscapes, may increase chances of colonisation (Mortimer et al. 1998; Shuey 2013) although these are sometimes not sufficient to guarantee colonist dispersal (e.g. Baum et al. 2004). Hence, accompanying habitat restoration with species reintroduction may be an essential strategy (Schultz et al. 2008; Jourdan et al. 2019). Pilot studies about the best methods to collect individuals and to reintroduce them with success (i.e. with the establishment of one population) should be undertaken.

- At a broad scale, the habitat restoration actions (including the creation of stepping stones) in order to increase *L. macrostigma* area of occupation, should be based on species distribution models that assess the species past area of occupation and identify where its reintroduction would be most relevant. Species distribution models should also take into account the effects of climate change (e.g. reduction of the risk of ice embedment of the eggs, yet earlier drying of temporary ponds increasing larval mortality) as these effects are likely to influence the species future distribution in apparently opposing ways. Further, the direct transfer of our findings in the Camargue area to other regions should be considered with care. For example, the odonates phenology varies across *L. macrostigma* distribution area and this likely influence the timing and duration of the hydroperiod favourable to the species. Also, *L. macrostigma* adults use in other regions other plant species to lay their eggs, for example *Schoenoplectus litoralis* which is abundant in Bulgaria and that we did not study here.

- Genetics can bring a relevant insight for the conservation practices targeting threatened species (e.g. Monroe & Britten 2014; see also Holderegger et al. 2019). Genetic assessments can reliably detect isolated populations facing particular threats, e.g. bottleneck events, genetic drift and an increased risk of inbreeding. First, at a global scale, conservation genetic studies may identify different genetic groups and populations requiring special attention and protection. In the context of reintroduction, the question of the source population(s) is critical. Indeed, maintaining or enhancing local genetic diversity using further population may increase local fitness (heterosis) or, to the opposite be against local adaptations (outbreeding depression) (e.g. Fountain et al. 2015). The genetic pattern of *L. macrostigma* at the global scale is currently under study. Second, the repeated population size drops due to unfavourable climatic (i.e. hydrologic) conditions some years, are likely to act as bottlenecks. However, population genetics and possible resilience (possibly based on a metapopulation functioning) at the local scale remain to explore.

- Habitat restoration in favour of *L. macrostigma*, or using the species as flagship for temporary brackish ponds, should be now undertaken at a larger scale along the Mediterranean coast, especially

but not exclusively. In this regard, the results of this PhD should guide future changes in conservation practices and pond creation (evidence-based conservation; see Sutherland et al. 2004). Yet, to provide empirical support of conservation recommendations, there is a need for more field experiments with monitoring describing ecological trajectories over the long-term (Wortley et al. 2013; Cooke et al. 2018; Zedler 2000). The relationship between the salinity levels of the soil, the ground water and the water of created pond is of particular interest. Further, in the context of climate change, long-term monitoring should allow an adaptive management approach, as current reference ecosystems are likely to represent increasingly irrelevant targets in a changing landscape (Shirk et al. 2022). Also, the management measures we suggest here in favour of *L. macrostigma* may benefit other species typical of temporary brackish ponds (including plants), but other species of conservation value may do worse under the suggested conditions. Hence, there is a need of a global biodiversity assessment in the restored/recreated ponds. At a larger geographic scale, there should be a need for mixture of pond types with a range of hydroregimes. Finally, long-term monitoring not only plays a crucial role in our understanding of restoration science but also allows the assessment of conservation policies and management strategies, in order to implement impactful projects in the future (Cooke et al. 2018; Romanelli et al. 2021). Hence, long-term monitoring of the reproductive success and abundance of *L. macrostigma* and other odonates, abundance of plants used by *L. macrostigma* during oviposition, water and salinity levels... in restored and created ponds would help identifying the causes of success (or failure) of conservation actions and making better predictions.



### Take-home messages

- This PhD aimed at understanding to better manage and then convey, as a crucial sequence in biodiversity conservation and a further advocacy for an evidence-based approach of conservation.
- An integrated life cycle approach, including the egg and larval stages, for the study of insect species' ecology is essential to undertake efficient conservation and restoration actions in their favour.
- Given insects extinction rate, such studies should target threatened species with priority to those that can be use as flagship for the conservation of threatened habitats.
- *Lestes macrostigma* shows life-history traits (egg resistance and hatching plasticity, fast growing and salinity tolerant larvae, oviposition substrate preference and plasticity) that are adapted to the unpredictability of the flooding conditions of temporary brackish waters, its typical habitat.
- To favour *Lestes macrostigma*, best management practices first consist in setting an optimal timing and duration of flooding of temporary brackish waterbodies, which – in the southern France – starts in November and lasts until June. Second, during this flooding period, “high” water levels (at least 30 cm in large shallow marshes and, even better, 80 cm in smaller deeper ponds) should be maintained. Salinity levels should not exceed 4–6 g/L at the time of hatching (mid-March – mid-April) and 10–12 g/L during the emergence period (in May). In the context of climate change, this may involve active water management using an irrigation network and eventually artificial pumping.
- The Sea club-rush *Bolboschoenus maritimus* and the Sea rush *Juncus maritimus*, respectively as preferred oviposition plant and of secondary choice for *Lestes macrostigma*, should be favoured in the existing temporary brackish waterbodies by an adapted grazing scheme. In created ponds, vegetation colonisation should be assisted by transferring seeds of aquatic plant and tubers of helophyte species to accelerate and maximise restoration success.
- Using *Lestes macrostigma* as a flagship, restoring existing habitats and creating new ponds of relative small size (200–1,000 m<sup>2</sup>) should be undertaken to provide a network of temporary brackish waterbodies which will benefit wetland biodiversity, whatever colonized by *L. macrostigma* or not.

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## Publications

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## Samenvatting

Insecten zijn de snelst afnemende soortengroep en behoren tot de soorten die het meest bedreigd worden door antropogene activiteiten. Ze worden echter grotendeels over het hoofd gezien in natuurbeschermingsbeleid en bijhorend wetenschappelijk onderzoek. Wetlands zijn de afgelopen eeuwen sterk achteruitgegaan en worden nog steeds sterk bedreigd, wat gevolgen heeft voor alle facetten van de biodiversiteit, inclusief insecten. Odonaten (waterjuffers en libellen) zijn waardevolle indicatoren voor de conservatie-status van wetlands. Habitatherstel (inclusief habitatrecreatie) is in het huidige decennium als prioritaire strategie naar voren geschoven. Dergelijke beschermingsacties worden vergemakkelijkt door gebruik te maken van vlaggenschipsoorten, maar om efficiënt te zijn is een grondige kennis van hun ecologische vereisten noodzakelijk. Deze doctoraatsthesis richtte zich op de bedreigde waterjuffer *Lestes macrostigma*, als vlaggenschipsoort om het behoud en herstel van tijdelijke brakke vijvers te implementeren. Er was beperkte kennis over de invloed van hydroperiodes en zoutgehaltes op eieren en larven, belangrijke stadia in de populatiedynamiek van odonaten. Met het doel om op feiten gebaseerde beschermingsacties te ondernemen, wilden we deze kennishiaten opvullen door vier studies uit te voeren in de Camargue, Zuid-Frankrijk. Dit gebied wordt gekenmerkt door zoutafzettingen en een mediterrane klimaat, waardoor brakke en tijdelijke waterrijke habitatten ontstaan die broedpopulaties van *L. macrostigma* herbergen.

Om onze kennis van het eistadium te verbeteren, bestudeerden we eerst de embryonale ontwikkeling en de fenologie en het ontluikingssucces in twee tijdelijke brakke vijvers. We stelden vast dat de eitjes – gelegd in plantenstengels tijdens de late lente – in een vroeg ontwikkelingsstadium bleven tijdens de zomer, herfst en winter. Dit verhoogt waarschijnlijk hun weerstand tegen extreme temperaturen en illustreert de aanpassing van *L. macrostigma* aan tijdelijke habitatten met een onzekere inundatieperiode. Toch vermindert de inbedding in ijs van de plantenstengels de overleving van de eitjes, wat suggereert dat hogere waterniveaus boven de stengels de eisterfte, veroorzaakt door lagere wintertemperaturen, zouden verminderen. Het ontluiken duurde van midden maart tot midden april en verliep synchroon. Verschillen in fenologie tussen de vijvers waren hoogstwaarschijnlijk te wijten aan verschillen in watertemperatuur.

Het zoutgehalte van water is een belangrijke structurerende kracht van insectengemeenschappen. Daarom bestudeerden we in het laboratorium het effect van zoutgehaltes tijdens

de ei- en larvestadia. We vonden geen effect van zeezout op de overleving van eieren (succes van ontluiken) in het bereik van 2-9,5 g/L. Daarentegen nam de overleving van larven toe met het zoutgehalte van 0,5 tot 8 g/L, maar werd het gereduceerd tot nul bij 16 g/L. Toenemende zoutgehaltes verlengden de ontwikkelingstijd tot het volwassen stadium, verlaagden de groeisnelheid van de larven en verminderden de grootte van de volwassen dieren bij het uitkomen. Het zoutgehalte in het larvestadium had geen effect op de spiermassa van de borstkas van de volwassen dieren, de vliegprestaties, de investering in immuunfunctie en de beschikbare energie. Het energieverbruik nam echter toe bij hogere zoutgehaltes, wat resulteerde in een lager netto energiebudget in het volwassen stadium. Onze resultaten gaven daarom aan dat *L. macrostigma* een zout-generalist is (fundamentele niche) waarvan de gerealiseerde niche verschoof naar brakke wateren.

Andere factoren, vooral biotische interacties, die optreden tijdens de larvale fase kunnen gevolgen hebben voor de larven en doorwerken naar de volwassen dieren. Daarom voerden we een veldstudie uit in 33 vijvers, waarbij we het effect van 14 biotische en abiotische variabelen op de abundantie van *L. macrostigma* larven en de grootte van de volwassen dieren onderzochten. We vonden dat larven talrijker waren in kleinere vijvers en vijvers met een latere inundatie in de herfst-winter. Dit leek veroorzaakt te worden door roofzuchtige libellenlarven, die talrijker en groter waren in vijvers met een vroegere inundatie. Een hoge gevoeligheid van de larven van *L. macrostigma* voor predatoren zou in verband kunnen worden gebracht met hun hoge activiteitsniveaus, die samenhangen met hun snelle groei en ontwikkeling als gevolg van het uitdrogen van hun tijdelijke habitatten. Verder waren adulten *L. macrostigma* meestal groter aan vijvers met een hoger waterpeil, waarschijnlijk omdat de lagere watertemperaturen de groeiperiode van de larven verlengen. Daarentegen vonden we geen effecten van het zoutgehalte van het water en de beschikbaarheid van ovipositieplanten op de abundantie van *L. macrostigma* larven.

Om te onderzoeken of de voorkeur van volwassen dieren voor bepaalde planten om hun eitjes te leggen ten goede komt aan de nakomelingen, bestudeerden we onder semi-gecontroleerde omstandigheden het effect van de inundatiedatum en het type plantenstengel waarin de eitjes werden gelegd op het ontluikingssucces. We ontdekten dat bevochtiging noodzakelijk was voor het uitkomen van de eieren. Eieren konden tegen uitdroging tot aan de inundatie van de vijver in april (d.w.z. na 10 maanden in droge omstandigheden), maar met een lage overlevingskans, terwijl latere inundaties leidden tot geen ontluiking. Eieren gelegd in de plant *Bolboschoenus maritimus* kenden een hogere overlevingskans en een snellere embryonale ontwikkeling. Aangezien *B. maritimus* in diepere delen van de vijver groeit en dus eerder overstroomd wordt, suggereert dit dat de voorkeur van volwassen dieren voor deze plant een adaptieve reactie is op het onzekere tijdstip van inundatie van de typische habitatten van *L. macrostigma*.

De resultaten van deze studies, die belangrijke fasen van de levenscyclus van *L. macrostigma* integreerden, leveren een wetenschappelijke basis om beschermingsacties te ontwikkelen die niet alleen gericht zijn op de soort, maar ook op het herstel van tijdelijke brakke vijvers in het Middellandse Zeegebied door *L. macrostigma* als vlaggenschipsoort te gebruiken. Het instellen van een hydroperiode van november tot juni is cruciaal om het risico op uitdroging van de eieren te verminderen en de larven van *L. macrostigma* te bevoordelen ten opzichte van die van andere odonaatsoorten in het studiegebied. Dit kan ecologische engineering vereisen op de schaal van het stroomgebied, gebaseerd op het modelleren van de hydrologie van de vijver met behulp van meteorologische en topografische gegevens. Gezien de klimaatverandering en de toenemende onvoorspelbaarheid van de neerslag in het Middellandse Zeegebied, kan het kunstmatig laten overstromen van sommige vijvers door irrigatie de duurzaamheid van een aantal vaste voortplantingsplaatsen garanderen. Irrigatie kan ook helpen om het zoutgehalte onder controle te houden en het waterniveau hoog te houden tijdens de inundatieperiode om de fitness van de soort te verhogen. De kolonisatie van nieuw aangelegde vijvers door *B. maritimus* moet worden versneld door in situ verzamelde knollen en stengels transplanteren. De bestaande vegetatie moet in stand worden gehouden door een aangepast begrazingsschema voor vee toe te passen dat gekoppeld is aan de beoogde hydroperiode. Gezien de achteruitgang van tijdelijke brakke vijvers in het verleden, moet een netwerk van deze habitatten worden hersteld of herschapen met *L. macrostigma* als vlaggenschipsoort. Het monitoren op lange termijn zou helpen bij het identificeren van de redenen van het succes of falen van een project en het verbeteren van het ontwerp van toekomstige projecten. In dit opzicht is nieuw onderzoek naar de herintroductie van *L. macrostigma* in herstelde vijvers noodzakelijk. Dit doctoraat biedt een belangrijke voorbeeldstudie over hoe men rekening kan houden met bedreigde insecten in strategieën voor het behoud van biodiversiteit.

## Résumé

Les insectes constituent le groupe d'espèces déclinant le plus rapidement et sont parmi les plus menacés par les activités humaines. Malgré cela, ils sont largement négligés par les politiques de conservation et la recherche en biologie de la conservation. Les zones humides ont fortement disparu durant le dernier siècle et demeurent à ce jour menacées, ce qui impacte tous les groupes taxonomiques de la biodiversité, dont les insectes. Les odonates (les demoiselles et les libellules) sont de bons indicateurs de l'état de conservation des zones humides. La restauration d'habitats (recréation incluse) a été érigée comme une stratégie prioritaire durant la décennie en cours. De telles actions de conservation sont facilitées par l'usage d'une espèce étendard ; mais pour être efficaces, une connaissance solide de leurs exigences écologiques est nécessaire. Cette thèse de doctorat s'est focalisée sur la demoiselle menacée *Lestes macrostigma*, en tant qu'espèce étendard pour la conservation et la restauration des mares temporaires saumâtres. Les connaissances étaient lacunaires quant à l'influence du régime hydrologique et du niveau de salinité de l'eau sur les œufs et les larves, qui sont pourtant des stades clés de la dynamique des populations d'odonates. Dans le but d'entreprendre des actions de conservation basées sur des preuves, nous avons taché de combler ces lacunes en menant quatre études en Camargue, dans le sud de la France. Cette zone géographique se caractérise par des dépôts de sel et un climat méditerranéen, qui favorise l'apparition de zones humides temporaires et saumâtres abritant des populations reproductrices de *L. macrostigma*.

Afin d'améliorer notre connaissance de la biologie de l'œuf, nous avons étudié le développement embryonnaire ainsi que la phénologie et le succès d'éclosion dans deux mares temporaires saumâtres. Nous avons trouvé que les œufs – pondus dans des tiges de plantes durant le printemps précédent – restent à un stade de développement embryonnaire précoce durant l'été, l'automne et l'hiver. Ceci augmente probablement leur résistance aux températures extrêmes et illustre l'adaptation de *L. macrostigma* aux milieux temporaires dont la temporalité de submersion est incertaine. Cependant, la prise dans la glace des tiges de plantes contenant les œufs diminue leur survie, suggérant que des niveaux d'eau plus hauts au-dessus des tiges réduirait la mortalité des œufs en les protégeant des plus basses températures hivernales. L'éclosion s'est étalée de mi-mars à mi-avril et a été très synchronisée. Les différences de phénologie entre les deux mares s'expliquent probablement par des différences de température de leurs eaux.

La salinité de l'eau est un facteur pilote majeur des communautés d'insectes. Aussi avons-nous étudié en conditions de laboratoire les effets des niveaux de salinité vécus durant les stades embryonnaire et larvaire. Nous n'avons pas trouvé d'effet de la concentration en sel de mer sur la survie des œufs (succès d'éclosion) dans la gamme 2–9.5 g/L. En revanche, la survie larvaire a augmenté avec le niveau de salinité entre 0,5 et 8 g/L mais a chuté à zéro à 16 g/L. Les niveaux de salinité croissants ont augmenté la durée de développement larvaire jusqu'à l'âge adulte, et ont diminué les taux de croissance ainsi que la taille des adultes à l'émergence. Le niveau de salinité vécu durant la phase larvaire n'a eu d'effet ni sur la masse de muscles thoraciques des adultes, ni sur leur aptitude au vol, ni sur leur investissement dans les fonctions immunitaires, ni sur l'énergie qui leur était disponible. Toutefois, la consommation énergétique des adultes a augmenté avec les niveaux de salinité, résultant en un bilan énergétique plus faible. Nos résultats ont donc indiqué que, par rapport à la salinité de l'eau, *L. macrostigma* est un généraliste (niche écologique fondamentale) dont la niche écologique réalisée s'est déplacée vers les eaux saumâtres.

D'autres facteurs, comme les interactions biologiques, vécues durant la phase larvaire peuvent avoir des effets sur les larves et se répercuter sur les adultes. Nous avons donc mené une étude de terrain sur 33 mares et marais, en évaluant l'effet de 14 variables biotiques et abiotiques sur l'abondance des larves de *L. macrostigma* et sur la taille des adultes à l'émergence. Nous avons trouvé que les larves étaient plus abondantes dans les mares de plus petite taille et dont les dates de submersion étaient plus tardives durant l'automne et l'hiver. Ceci semblait s'exprimer par les larves prédatrices de libellules de la famille des Aeshnidae, qui étaient plus grandes et plus abondantes dans les mares à submersion plus précoce. Une grande vulnérabilité des larves de *L. macrostigma* aux prédateurs pourrait être liée à un haut niveau d'activité, qui serait associé à une croissance rapide et un fort taux de développement qu'impose l'assèchement de leur habitat temporaire. En outre, les adultes de *L. macrostigma* ont eu tendance à être plus grands lorsqu'ils émergeaient de mares ayant de plus hauts niveaux d'eau, probablement du fait que les eaux plus froides allongent la période de développement larvaire. En revanche, nous n'avons trouvé aucun effet des niveaux de salinité ni de la disponibilité en plantes hôtes de ponte sur l'abondance des larves de *L. macrostigma*.

Afin d'évaluer si la préférence que montrent les adultes pour certains types de plantes lors de la ponte sont bénéfiques à leur progéniture, nous avons étudié en conditions semi-contrôlées les effets de la date de submersion des pontes et du type de plante dans lequel les œufs ont été pondus sur leur succès d'éclosion. Nous avons trouvé que la submersion des pontes est nécessaire à l'éclosion. Les œufs ont été capables de supporter – bien qu'avec un faible taux de survie – la sécheresse jusqu'au mois d'avril (c'est-à-dire 10 mois sans submersion), mais aucun œuf n'a pu éclore dans le cas d'une submersion plus tardive. Les œufs pondus dans le Scirpe maritime *Bolboschoenus maritimus* ont eu un

taux de survie plus grand et un développement embryonnaire plus rapide. Puisque *B. maritimus* pousse dans des parties des mares plus profondes, et est par conséquent submergé plus tôt que les autres plantes hôtes de ponte, cela suggère que la préférence que montrent les adultes pour *B. maritimus* lors de la ponte est une réponse adaptative à l'incertitude des conditions de submersion de l'habitat typique de *L. macrostigma*.

Les résultats de ces études, qui intègrent les stades clés du cycle de vie de *L. macrostigma*, fournissent une base scientifique sur laquelle développer les actions de conservation visant non seulement l'espèce, mais aussi la restauration de mares méditerranéennes temporaires saumâtres en utilisant *L. macrostigma* comme espèce étendard. La mise en place d'une hydropériode s'étendant de novembre à juin est cruciale pour réduire le risque de dessiccation de ses œufs et pour favoriser ses larves par rapport à celles d'autres espèces d'odonates dans la région étudiée. Ceci pourrait nécessiter des travaux d'ingénierie écologique à l'échelle du bassin versant des mares, basés sur la modélisation de leur hydrologie en utilisant des données météorologiques et topographiques. Compte tenu des changements climatiques, et notamment de l'augmentation de l'imprévisibilité des précipitations dans la zone méditerranéenne, la mise en eau artificielle par un réseau d'irrigation pourrait être la garantie du maintien d'un nombre minimal de sites de reproduction pérennes pour l'espèce. L'irrigation pourrait également aider à contrôler les niveaux de salinités et à maintenir des niveaux d'eau élevés durant la phase inondée afin d'augmenter la valeur sélective des individus. La colonisation des mares créées par *B. maritimus* devrait être accélérée par la transplantation de tubercules et de rhizomes collectés sur le terrain. La végétation existante devrait être entretenue par la mise en place d'un pâturage dont le calendrier et la pression soient liés au régime hydrologique visé. Compte tenu de la dégradation passée des milieux temporaires saumâtres, un réseau de mares de ce type devrait être restauré ou recréé en utilisant *L. macrostigma* comme espèce étendard. Leur suivi sur le long terme aiderait à identifier les facteurs de succès ou d'échec de ces actions de conservation, et à améliorer la conception des projets à venir. À cet égard, de nouvelles recherches portant sur la réintroduction de *L. macrostigma* dans certaines mares restaurées sont nécessaires. Cette thèse représente une importante étude de cas sur la manière de prendre en considération les insectes menacés dans les stratégies de conservation de la biodiversité.



## Resumo

Os insetos são o grupo de espécies com declínio mais rápido e estão entre os mais ameaçados pelas atividades antrópicas. No entanto, eles são amplamente negligenciados nas políticas de conservação e na investigação sobre conservação. As zonas húmidas diminuíram bastante ao longo dos últimos séculos e continuam a enfrentar ameaças importantes, com impacto em todos os setores da biodiversidade, incluindo os insetos. Os odonatos (libélulas e libelinhas) são indicadores valiosos do estado de conservação das zonas húmidas. A restauração de habitats (incluindo a criação de habitats) é considerada uma estratégia prioritária durante a presente década. Tais ações de conservação são promovidas utilizando espécies bandeira emblemáticas, mas para serem eficientes é necessário um conhecimento profundo das suas exigências ecológicas. Esta tese de doutoramento centrou-se na ameaçada libelinha Lestes-das-Marismas (*Lestes macrostigma*), como espécie bandeira para implementar medidas de conservação e restauro de lagoas salobras temporárias. Havia conhecimento limitado sobre a influência dos regimes hídricos e dos níveis de salinidade da água nos ovos e larvas, que são fases-chave na dinâmica populacional de odonatos. Com o objetivo de realizar ações de conservação baseadas em evidências, pretendemos preencher estas lacunas de conhecimento através da realização de quatro estudos na região de Camarga, no sul da França. Esta região é caracterizada pela presença de depósitos de sal e um clima mediterrânico, criando habitats de zonas húmidas salobras e temporárias que albergam populações reprodutoras de Lestes-das-Marismas.

Para melhorar nosso conhecimento sobre a biologia dos ovos, estudamos o desenvolvimento embrionário, a fenologia e o sucesso da eclosão em dois viveiros de águas salobras temporárias. Descobrimos que os ovos – depositados nos rebentos das plantas durante o final da primavera – permaneceram numa fase inicial de desenvolvimento durante o verão, outono e inverno. Isto provavelmente aumenta a sua resistência a temperaturas extremas e ilustra a adaptação desta espécie a habitats temporários com tempos de inundação incertos. No entanto, a submersão dos rebentos das plantas no gelo diminuiu a sobrevivência dos ovos, sugerindo que níveis de água acima dos rebentos reduziram a mortalidade dos ovos causada pelas temperaturas mais baixas do inverno. A eclosão teve lugar desde meados de março a meados de abril e foi sincronizada. As diferenças na fenologia de eclosão entre os tanques foram provavelmente devidas a diferenças nas temperaturas da água.

A salinidade da água é um dos principais condicionadores das comunidades de insetos. Assim, estudamos em laboratório o efeito de distintos níveis de salinidade durante os estágios de ovo e larva.

Não encontramos nenhum efeito da salinidade na sobrevivência dos ovos (sucesso de eclosão) na faixa de 2 a 9,5 g/L. Por outro lado, a sobrevivência das larvas aumentou com o nível de salinidade de 0,5 para 8 g/L, mas foi reduzida a zero a 16 g/L. O aumento dos níveis de salinidade aumentou o tempo de desenvolvimento até a fase adulta, diminuiu as taxas de crescimento das larvas e reduziu o tamanho do adulto. O nível de salinidade testado na fase larvar não teve efeito na massa muscular do tórax adulto, no desempenho de voo, no investimento na função imunológica e na energia disponível. No entanto, o consumo de energia aumentou com níveis de salinidade mais elevados, resultando num menor saldo energético líquido na fase adulta. Os resultados indicaram, portanto, que Lestes-das-Marismas é um generalista salino (nicho fundamental) cujo nicho realizado deslocou-se para águas salobras.

Outros fatores, especialmente as interações bióticas durante a fase larvar podem ter consequências nas larvas e afetar os adultos. Por esse motivo, conduzimos um estudo de campo em 33 lagoas, testando o efeito de 14 variáveis bióticas e abióticas na abundância de larvas de Lestes-das-Marismas e no tamanho dos adultos na emergência. Descobrimos que as larvas eram mais abundantes em lagoas menores e com inundações posteriores no outono-inverno. Essa variação parece estar relacionada com a presença de larvas de libélulas de tira-olhos (Aeshnidae), que eram mais abundantes e maiores em lagoas inundadas anteriormente ao período de outono-inverno. Uma elevada suscetibilidade das larvas de Lestes-das-Marismas aos predadores pode estar relacionada com os seus elevados níveis de atividade associados ao seu rápido crescimento e taxa de desenvolvimento imposta pela secagem dos seus habitats temporários. Além disso, os indivíduos de Lestes-das-Marismas tendem a ser maiores à data de emergência em tanques com níveis de água mais elevados, muito provavelmente porque as temperaturas mais baixas da água prolongam o período de crescimento larvar. Por outro lado, não encontramos efeitos dos níveis de salinidade da água e da disponibilidade de plantas onde oviposita na abundância larvar de Lestes-das-Marismas.

Para investigar se a preferência dos adultos por determinadas plantas para depositar os seus ovos beneficia a sua descendência, utilizamos condições semicontroladas para estudar o efeito no sucesso da eclosão de acordo com a data da inundação e o tipo de rebento da planta onde os ovos foram depositados. Descobrimos que a submersão em água era obrigatória para a ocorrência de eclosão. Os ovos foram capazes de resistir à dessecação até às inundações, no final de abril (ou seja, após 10 meses em condições não inundadas) – embora com baixa taxa de sobrevivência – mas as inundações posteriores não resultaram em eclosão. A postura de ovos no junco marinho *Bolboschoenus maritimus* proporcionou maior taxa de sobrevivência e desenvolvimento embrionário mais rápido. Dado que *B. maritimus* cresce nas partes mais profundas da lagoa, sofrendo assim

inundações mais precoces, sugere que a preferência dos adultos por esta planta é uma resposta adaptativa às condições incertas de inundação do habitat típico de Lestes-das-Marismas.

Os resultados destes estudos, que integraram fases-chave do ciclo de vida de Lestes-das-Marismas, fornecem uma base científica para desenvolver ações de conservação visando não só a espécie, mas também o restauro de lagoas salobras temporárias do Mediterrâneo utilizando Lestes-das-Marismas como espécie bandeira. Definir um hidroperíodo de novembro a junho é crucial para reduzir os riscos de dessecação dos ovos e para favorecer as suas larvas em detrimento de outras espécies odonatos na região de estudo. Isto pode exigir engenharia ecológica à escala da bacia hidrográfica, baseada na modelação da hidrologia da lagoa utilizando dados meteorológicos e topográficos. Dadas as alterações climáticas e o aumento da imprevisibilidade das chuvas na zona mediterrânica, a inundação artificial de alguns charcos através da irrigação pode garantir a sustentabilidade de vários locais de reprodução permanentes. A irrigação também pode ajudar a controlar os níveis de salinidade e manter níveis elevados de água durante o período de cheias, a fim de aumentar a aptidão das espécies. A colonização de tanques recém-criados por *B. maritimus* deve ser acelerada através do transplante de tubérculos e rebentos colhidos *in situ*. A cobertura vegetal existente deve ser mantida através da implementação de um esquema de pastoreio adaptativo para o gado ligado ao regime hidrológico alvo. Considerando a degradação passada de lagoas salobras temporárias, uma rede destes habitats deve ser restaurada ou recriada usando Lestes-das-Marismas como espécie bandeira. A sua monitorização a longo prazo ajudaria a identificar as razões do sucesso ou fracasso do projeto e a melhorar a conceção de projetos futuros. Nesse sentido, são necessárias novas pesquisas sobre a reintrodução de Lestes-das-Marismas em alguns viveiros restaurados. Este doutoramento fornece um importante caso de estudo sobre como incluir insetos ameaçados nas estratégias de conservação da biodiversidade.

Tradução: Sónia Ferreira

## Resumen

Los insectos son el grupo de especies que está sufriendo un declive más rápido, y uno de los más amenazados por las actividades humanas. A pesar de ello, apenas han sido tenidos en cuenta en la investigación y las políticas sobre conservación. Los humedales, que han visto reducidos en gran medida su número y extensión a lo largo de los últimos siglos, continúan afrontando importantes amenazas que afectan a todos los componentes de la biodiversidad, incluyendo los insectos. Los odonatos (libélulas y caballitos del diablo) son valiosos indicadores del estado de conservación de los humedales. La restauración del hábitat (incluyendo la recreación de nuevos hábitats) se ha planteado como una estrategia prioritaria durante la década actual. Dichas acciones de conservación se ven respaldadas por el uso de especies bandera, pero para que sean eficaces es necesario un conocimiento profundo de sus requisitos ecológicos. Esta tesis doctoral se centró en el caballito amenazado *Lestes macrostigma* como especie bandera para llevar a cabo la conservación y restauración de humedales salobres temporales. A pesar de que las fases de huevo y larva son claves para la dinámica poblacional de los odonatos, el conocimiento sobre la influencia del régimen hídrico y los niveles de salinidad del agua en dichas etapas era limitado. Con el objetivo de emprender acciones de conservación basadas en evidencias, nos propusimos reducir estas lagunas de conocimiento realizando cuatro estudios en la Camarga (sur de Francia). Esta zona se caracteriza por la presencia de depósitos de sal y un clima mediterráneo, que dan lugar a humedales salobres y temporales que albergan poblaciones reproductoras de *L. macrostigma*.

Para mejorar nuestro conocimiento de la biología durante la fase de huevo, estudiamos el desarrollo embrionario y la fenología y el éxito de la eclosión en dos lagunas temporales salobres. Encontramos que los huevos, que habían sido insertados en el interior de los tallos de las plantas al final de la primavera, permanecieron en una fase temprana de desarrollo durante el verano, el otoño y el invierno. Esto probablemente aumenta su resistencia a las temperaturas extremas e ilustra la adaptación de *L. macrostigma* a hábitats temporales en los que el momento de la inundación es incierto. Sin embargo, la congelación de los tallos de las plantas que contenían huevos disminuyó la supervivencia de estos, sugiriendo que el ascenso del nivel de agua por encima de los tallos reduciría la mortalidad de los huevos al protegerlos de las bajas temperaturas invernales. La eclosión se observó desde mediados de marzo hasta mediados de abril y fue sincronizada. Las diferencias observadas en

la fenología de la eclosión entre lagunas se debieron probablemente a las diferencias en la temperatura del agua.

La salinidad del agua es un factor determinante fundamental para las comunidades de insectos. Por ello, estudiamos en el laboratorio el efecto de los niveles de salinidad en las etapas de huevo y larva. No encontramos ningún efecto de la salinidad sobre la supervivencia del huevo (éxito de eclosión) en el rango de 2 a 9,5 g/l de sal. Por el contrario, la supervivencia de las larvas aumentó con el nivel de salinidad de 0,5 a 8 g/l pero se redujo a cero con salinidades de 16 g/l. A medida que se incrementó la salinidad, aumentó el tiempo de desarrollo hasta la fase adulta, disminuyó la tasa de crecimiento larvario y se redujo el tamaño de los adultos en el momento de la emergencia. El nivel de salinidad experimentado durante la fase larvaria no tuvo ningún efecto sobre la masa muscular del tórax del adulto, el rendimiento del vuelo, la inversión en la función inmunitaria y la energía disponible. Sin embargo, el consumo de energía aumentó con niveles de salinidad más elevados, dando lugar a un coste energético neto menor en la fase adulta. Nuestros resultados por tanto indican que *L. macrostigma*, en relación a la salinidad, es una especie generalista (nicho fundamental) cuyo nicho ecológico se ha desplazado hacia aguas salobres.

Otros factores -especialmente las interacciones bióticas- experimentados durante la fase larvaria pueden terminar por afectar a los adultos. Por ello, realizamos un experimento de campo en 33 lagunas, analizando el efecto de 14 variables bióticas y abióticas sobre la abundancia de larvas de *L. macrostigma* y el tamaño de los adultos en el momento de la emergencia. Encontramos que las larvas eran más abundantes en las lagunas más pequeñas que se inundaban más tardíamente en otoño-invierno. Esto parece estar relacionado con las larvas de ésnidos, que eran más abundantes y grandes en las charcas que se inundaban antes. La alta vulnerabilidad de las larvas de *L. macrostigma* a los depredadores podría estar relacionada con sus altos niveles de actividad asociados a los rápidos ritmos de crecimiento y desarrollo que impone la desecación de los hábitats temporales. Además, los adultos de *L. macrostigma* tendían a ser de mayor tamaño cuando emergían en lagunas con niveles altos de agua, muy probablemente porque la menor temperatura del agua prolonga el periodo de crecimiento larvario. Por el contrario, no encontramos efectos de los niveles de salinidad del agua ni de la disponibilidad de las plantas adecuadas para la puesta sobre la abundancia de larvas de *L. macrostigma*.

Para investigar si la preferencia que tienen los adultos por realizar sus puestas en determinadas plantas beneficiaba a su progenie, realizamos un experimento en condiciones semi controladas para estudiar el efecto del éxito de la eclosión según la fecha de inundación y el tipo de planta donde se habían insertado los huevos. Encontramos que la presencia de agua era imprescindible para que

tuviera lugar la eclosión. Los huevos fueron capaces de permanecer viables -aunque con bajas tasas de supervivencia- en ausencia de inundación hasta el mes de abril (es decir, 10 meses sin inundación), pero con fechas más tardías de inundación la eclosión no se produjo. Los huevos depositados en la castañuela (*Bolboschoenus maritimus*) tuvieron una mayor tasa de supervivencia y un desarrollo embrionario más rápido. Dado que la castañuela crece en las partes más profundas de la charca, experimentando por tanto una inundación más temprana, estos resultados sugieren que la preferencia de los adultos por esta planta es una respuesta adaptativa a las inciertas condiciones de inundación del hábitat típico de *L. macrostigma*.

Los resultados de estos estudios, que se desarrollaron sobre etapas clave del ciclo vital de *L. macrostigma*, proporcionan una base científica para llevar a cabo acciones de conservación dirigidas no solo a esta especie, sino también a la restauración de humedales salobres temporales mediterráneos utilizando *L. macrostigma* como especie bandera. Establecer un hidroperiodo de noviembre a junio es crucial para reducir los riesgos de desecación de los huevos y para favorecer a las larvas frente a las de otras especies de odonatos en el área de estudio. Para ello podrían ser necesarios trabajos de ingeniería ecológica a escala de cuenca, basados en la modelización de su hidrología a partir de datos meteorológicos y topográficos. Teniendo en cuenta el cambio climático y, en particular, la mayor imprevisibilidad de las precipitaciones en la zona mediterránea, la inundación artificial de algunas charcas mediante regadío podría garantizar el mantenimiento de un número mínimo de sitios fijos de reproducción. El riego también podría ayudar a controlar los niveles de salinidad y a mantener niveles de agua altos durante el periodo de inundación con el fin de aumentar la eficacia biológica de los individuos. En lagunas de nueva creación, la colonización por parte de *B. maritimus* debería acelerarse mediante el trasplante de rizomas y esquejes recogidos *in situ*. La cubierta vegetal existente debería mantenerse mediante la aplicación de un sistema de pastoreo adaptable, vinculado al régimen hidrológico previsto. Dada la degradación de las lagunas salobres temporales en el pasado, debería restaurarse o recrearse una red de estos hábitats utilizando *L. macrostigma* como especie bandera. Un seguimiento a largo plazo ayudaría a identificar los factores que explican el éxito o el fracaso de estas acciones de conservación y a mejorar el diseño de futuros proyectos. En este sentido, es necesario seguir investigando sobre la reintroducción de *L. macrostigma* en lagunas restauradas. Esta tesis representa un importante caso de estudio sobre cómo tener en cuenta a los insectos amenazados en las estrategias de conservación de la biodiversidad.

Traducción: Cecilia Díaz Martínez & Carmen Díaz-Paniagua

## Zusammenfassung

Insekten sind die am schnellsten rückläufige Artengruppe und gehören zu den am stärksten durch anthropogene Aktivitäten bedrohten Arten. Sie werden jedoch in der Naturschutzpolitik und der Naturschutzforschung weitgehend vernachlässigt. Feuchtgebiete sind in den letzten Jahrhunderten stark zurückgegangen und sind weiterhin erheblichen Bedrohungen ausgesetzt, die sich auf alle Bereiche der Artenvielfalt, einschließlich Insekten, auswirken. Odonaten (Klein- und Großlibellen) sind wertvolle Indikatoren für den Erhaltungszustand von Feuchtgebieten. Die Wiederherstellung von Lebensräumen (einschließlich der **Regeneration von Lebensräumen**) wurde im laufenden Jahrzehnt als vorrangige Strategie genannt. Solche Erhaltungsmaßnahmen werden durch den Einsatz von Zeigerarten erleichtert. Dazu ist eine gründliche Kenntnis ihrer ökologischen Anforderungen erforderlich, um effizient zu sein. Diese Doktorarbeit konzentrierte sich auf die bedrohte Kleinlibelle *Lestes macrostigma* als Zeigerart für die Erhaltung und Wiederherstellung temporärer Brackwasserteiche. Es gab nur begrenzte Kenntnisse über den Einfluss von Hydroregime und Salzgehalt des Wassers auf Eier und Larven, die Schlüsselstadien in der Dynamik der Libellenpopulation sind. Mit dem Ziel, evidenzbasierte Naturschutzmaßnahmen durchzuführen, wollten wir diese Wissenslücken durch die Durchführung von vier Studien in der Camargue in Südfrankreich schließen. Dieses Gebiet zeichnet sich durch Salzablagerungen und ein mediterranes Klima aus, wodurch brackige und temporäre Feuchtgebiete entstehen, in denen *L. macrostigma*-Brutpopulationen leben.

Um unser Wissen über die Eiologie zu verbessern, untersuchten wir die Embryonalentwicklung, die Phänologie und den Erfolg des Schlüpfens in zwei temporären Brackwasserteichen. Wir fanden heraus, dass die Eier, die im Spätfrühling in Pflanzensprossen gelegt wurden, im Sommer, Herbst und Winter in einem frühen Entwicklungsstadium blieben. Dies erhöht wahrscheinlich ihre Widerstandsfähigkeit gegenüber extremen Temperaturen und veranschaulicht die Anpassung von *L. macrostigma* an temporäre Lebensräume mit ungewissem Zeitpunkt der Überschwemmung. Allerdings verringert eine Vereisung der Pflanzensprosse die Überlebensrate der Eier, was darauf hindeutet, dass ein höherer Wasserstand oberhalb der Eilagen die durch niedrigere Wintertemperaturen verursachte Eiersterblichkeit verringern würde. Der Schlüpfvorgang dauerte von Mitte März bis Mitte April und erfolgte zeitsynchron. Unterschiede in der Schlüpfphänologie zwischen Teichen waren höchstwahrscheinlich auf Unterschiede in der Wassertemperatur zurückzuführen.

Der Salzgehalt des Wassers ist ein wesentlicher Faktor für Insektengemeinschaften. Daher haben wir im Labor die Auswirkungen des Salzgehalts im Ei- und Larvenstadium untersucht. Wir fanden keinen Einfluss von Meersalz auf das Überleben der Eier (Schlupferfolg) im Bereich von 2–9,5 g/l. Im Gegensatz dazu stieg die Überlebensrate der Larven mit einem Salzgehalt von 0,5 auf 8 g/l, sank jedoch bei 16 g/l auf null. Ein steigender Salzgehalt verlängerte die Entwicklungszeit bis zum Erwachsenenstadium, verringerte die Wachstumsraten der Larven und verringerte die Größe der Imagines beim Schlüpfen. Der Salzgehalt im Larvenstadium hatte keinen Einfluss auf die Brustmuskelmasse, die Flugleistung, die Investitionen in die Immunfunktion und die verfügbare Energie des erwachsenen Tieres. Allerdings stieg der Energieverbrauch mit höherem Salzgehalt, was zu einem geringeren Nettoenergiehaushalt im Erwachsenenstadium führte. Unsere Ergebnisse deuten daher darauf hin, dass *L. macrostigma* ein Generalist bezüglich Salzwasser (grundlegende Nische) ist, dessen realisierte Nische sich in Richtung Brackwasser verlagert.

Andere Faktoren, insbesondere biotische Wechselwirkungen, die im Larvenstadium auftreten, können Einflüsse auf die Larven haben und sich auf die Imagines auswirken. Wir führten daher eine Feldstudie in 33 Teichen durch und testeten die Auswirkung von 14 biotischen und abiotischen Variablen auf die Larvenhäufigkeit und die Größe erwachsener *L. macrostigma*-Larven beim Schlupf. Wir fanden heraus, dass die Larven in kleineren Teichen und bei späteren Überschwemmungen im Herbst und Winter häufiger vorkommen. Dies schien durch räuberische Aeshniden-Libellenlarven verursacht zu werden, die in früher überfluteten Teichen häufiger und größer waren. Eine hohe Anfälligkeit der *L. macrostigma*-Larven gegenüber Fressfeinden könnte mit ihrem hohen Aktivitätsniveau zusammenhängen, das mit ihrer schnellen Wachstums- und Entwicklungsrate verbunden ist, die durch die Austrocknung ihrer temporären Lebensräume verursacht wird. Darüber hinaus wiesen *L. macrostigma* Imagines, die aus Teichen mit höherem Wasserstand schlüpften, eine höhere Körpergröße auf, was höchstwahrscheinlich darauf zurückzuführen ist, dass die niedrigeren Wassertemperaturen die Wachstumsphase der Larven verlängern. Im Gegensatz dazu fanden wir keine Auswirkungen des Salzgehalts des Wassers und der Verfügbarkeit von Eiablagepflanzen auf die Larvenhäufigkeit von *L. macrostigma*.

Um zu untersuchen, ob die Vorliebe der Imagines für die Eiablage in bestimmte Pflanzen für die Nachkommen von Nutzen ist, haben wir unter semikontrollierten Bedingungen die Auswirkung auf den Schlupferfolg abhängig vom Überschwemmungsdatum und der Eiablagepflanze untersucht. Wir fanden heraus, dass eine Befeuchtung der Eier zum Schlüpfen zwingend erforderlich ist. Die Eier konnten ein Trockenfallen bis zur Überschwemmung erst im April (d. h. nach 10 Monaten ohne Überschwemmung) verkraften – allerdings mit geringer Überlebensrate –, spätere Überschwemmungen führten jedoch zu keinem Schlupf. Das Legen von Eiern in der Strandsimse *Bolboschoenus maritimus* sorgte für eine



höhere Überlebensrate und eine schnellere Embryonalentwicklung. Angesichts der Tatsache, dass *B. maritimus* in tieferen Teilen des Teichs wächst und daher früher Überschwemmungen erleidet, lässt sich vermuten, dass die Bevorzugung dieser Pflanze durch Imagines eine adaptive Reaktion auf die unsicheren Überschwemmungsbedingungen des typischen Lebensraums von *L. macrostigma* ist.

Die Ergebnisse dieser Studien, die wichtige Phasen des Lebenszyklus von *L. macrostigma* umfassten, bieten eine wissenschaftliche Grundlage für die Entwicklung von Schutzmaßnahmen, die nicht nur auf die Art abzielen, sondern auch auf die Wiederherstellung temporärer Brackwasserteiche im Mittelmeerraum, wenn *L. macrostigma* als Zeigerart verwendet wird. Die Festlegung einer Wasserführung von November bis Juni ist von entscheidender Bedeutung, um das Risiko einer Austrocknung der Eier zu verringern und ihre Larven gegenüber denen anderer Libellenarten in der Untersuchungsregion zu begünstigen. Dies kann eine ökologische Planung auf der Ebene des Wassereinzugsgebiets erfordern, die auf der Modellierung der Teichhydrologie unter Verwendung meteorologischer und topografischer Daten basiert. Angesichts des Klimawandels und der zunehmenden Unvorhersehbarkeit von Niederschlägen im Mittelmeerraum können durch künstliche Überflutung von Teichen mehrjährige Fortpflanzungsbiotope nachhaltig stabilisiert werden. Bewässerung kann auch dazu beitragen, den Salzgehalt zu kontrollieren und während der Überschwemmungsperiode einen hohen Wasserstand aufrechtzuerhalten, um die Fitness der Art zu verbessern. Die Besiedlung neu angelegter Teiche durch *B. maritimus* sollte durch die Umpflanzung von vor Ort gesammelten Diasporen beschleunigt werden. Die bestehende Vegetationsdecke sollte durch die Umsetzung eines adaptiven Beweidungssystems durch Nutztiere, die an das angestrebte Hydroregime gekoppelt sind, erhalten bleiben. In Anbetracht des Rückgangs und der Zerstörung temporärer Brackwasserteiche in der Vergangenheit sollte ein Netzwerk dieser Lebensräume wiederhergestellt oder neu geschaffen werden, wobei *L. macrostigma* als Zeigerart verwendet werden sollte. Ihre langfristige Überwachung würde dazu beitragen, die Gründe für den Erfolg oder Misserfolg des Projekts zu ermitteln und die Gestaltung zukünftiger Projekte zu verbessern. In diesem Zusammenhang sind neue Untersuchungen zur Wiederansiedelung von *L. macrostigma* an einigen restaurierten Teichen erforderlich. Diese Doktorarbeit liefert eine wichtige Fallstudie darüber, wie bedrohte Insekten in Strategien zum Schutz der biologischen Vielfalt berücksichtigt werden können.

Übersetzung: Theodor Benken

## Sintesi

Tra tutti gli animali, gli insetti sono quelli in più rapido declino e tra i più minacciati dalle attività antropiche. Infatti, sono stati ampiamente trascurati nelle politiche di conservazione e nella ricerca sullo stato di conservazione. Le zone umide sono diminuite molto durante gli ultimi secoli e continuano ad essere minacciate, con ripercussioni importanti su tutta la biodiversità che ospitano, compresi gli insetti. Gli odonati (damigelle e libellule) sono preziosi indicatori dello stato di conservazione delle zone umide. Il ripristino degli habitat (compresa la creazione di nuovi habitat) è considerata come una strategia prioritaria nel decennio in corso. Tali azioni di conservazione sono facilitate dall'utilizzo di specie ombrello, ma per essere efficaci è necessaria una conoscenza approfondita delle loro esigenze ecologiche. Questa tesi di dottorato si è concentrata sulla damigella *Lestes macrostigma*, una specie minacciata e specie ombrello per la conservazione e per il ripristino degli stagni salmastri temporanei. Fino ad oggi le conoscenze sull'influenza del regime idrico e dei livelli di salinità dell'acqua sulle uova e sulle larve, che sono stadi chiave nella dinamica delle popolazioni di odonati, erano limitate. Con l'obiettivo di intraprendere azioni di conservazione basate su dati certi, abbiamo lavorato per colmare queste lacune conoscitive conducendo quattro studi in Camargue, nella Francia meridionale. Quest'area è caratterizzata da depositi salini e da un clima mediterraneo, che insieme creano habitat salmastri e zone umide temporanee che ospitano popolazioni riproduttive di *L. macrostigma*.

Per migliorare le nostre conoscenze sulla biologia delle uova, abbiamo studiato lo sviluppo embrionale, la fenologia e il successo della schiusa in due stagni salmastri temporanei. Abbiamo scoperto che le uova – deposte nei fusti delle piante in tarda primavera – sono rimaste in uno stadio di sviluppo precoce durante l'estate, l'autunno e l'inverno. Questo probabilmente aumenta la loro resistenza alle temperature estreme e illustra l'adattamento di *L. macrostigma* agli habitat temporanei con periodi di allagamenti incerti. Tuttavia, se il fusto viene inglobato nel ghiaccio, la sopravvivenza delle uova diminuisce e questo suggerisce che livelli alti d'acqua, che coprono i fusti, associati alle basse temperature invernali diminuisce la mortalità delle uova. La schiusa è avvenuta da metà marzo a metà aprile ed era sincronizzata. Le differenze nella fenologia della schiusa osservate tra gli stagni sono probabilmente da attribuire alle diverse temperature dell'acqua.

La salinità dell'acqua è un fattore determinante per le comunità di insetti. Per questo motivo, abbiamo studiato in laboratorio l'effetto dei livelli di salinità a cui sono esposte le uova e le larve. Non

abbiamo riscontrato nessuno effetto del sale marino sulla sopravvivenza delle uova (successo della schiusa) nell'intervallo 2-9,5 g/L. Al contrario, la sopravvivenza delle larve è aumentata con una salinità tra 0,5 e 8 g/L, ma si è ridotta a zero a 16 g/L. L'aumento dei livelli di salinità ha aumentato quindi, il tempo di sviluppo fino allo stadio adulto ma ha diminuito i tassi di crescita larvale e ridotto le dimensioni degli adulti dopo lo sfarfallamento. Il livello di salinità dell'acqua durante lo stadio larvale non ha avuto alcun effetto sulla massa muscolare del torace degli adulti, sulle capacità di volo, sull'investimento nella funzione immunitaria e sull'energia disponibile. Tuttavia, il consumo di energia é aumentato con livelli di salinità più elevati, determinando un bilancio energetico netto inferiore nello stadio adulto. I nostri risultati indicano quindi che *L. macrostigma* è un generalista salino (nicchia fondamentale), la cui nicchia ecologica realizzata si è spostata verso le acque salmastre.

Altri fattori, tra cui soprattutto le interazioni biotiche a cui sono state esposte le larve, possono avere influenzato le larve ripercuotendosi poi sugli adulti. Abbiamo quindi condotto uno studio sul campo in 33 stagni, verificando l'effetto di 14 variabili biotiche e abiotiche sull'abbondanza di larve di *L. macrostigma* e sulle dimensioni degli adulti allo sfarfallamento. Abbiamo scoperto che le larve erano più abbondanti negli stagni più piccoli e con inondazioni più tardive in autunno-inverno. Questo sembra essere causato dalle larve predatrici di Aeshnidae, che erano più abbondanti e più grandi negli stagni inondati precedentemente. L'alta suscettibilità delle larve di *L. macrostigma* a questi predatori potrebbe essere legata ai loro elevati livelli di attività associati alla crescita rapida e al tasso di sviluppo, caratteristiche imposte dal prosciugarsi dei loro habitat temporanei. Inoltre, *L. macrostigma* sfarfallati da stagni più grandi e con livelli d'acqua più alti, erano più grandi, molto probabilmente perché le temperature più basse dell'acqua prolungano il periodo di crescita larvale. Invece, non abbiamo riscontrato nessun effetto dei livelli di salinità dell'acqua e della disponibilità di piante per l'ovodeposizione sull'abbondanza larvale di *L. macrostigma*.

Per verificare se la preferenza degli adulti per determinate piante per la deposizione delle uova avesse un effetto sulle larve, abbiamo utilizzato condizioni semi-controllate per studiare l'effetto sul successo della schiusa in base alla data di inondazione e al tipo di fusto della pianta in cui sono state deposte le uova. Abbiamo scoperto che la presenza di acqua era obbligatoria per la schiusa. Le uova sono state in grado di sopportare l'assenza dell'acqua fino all'allagamento di aprile (cioè dopo 10 mesi in condizioni non allagate), ma con un basso tasso di sopravvivenza, mentre l'allagamento successivo non ha portato alla schiusa. La deposizione delle uova nello scirpo marittimo *Bolboschoenus maritimus* ha mostrato in un tasso di sopravvivenza più elevato e uno sviluppo embrionale più rapido. Il fatto che *B. maritimus* cresca in zone più profonde dello stagno, e che quindi subisca inondazioni più precoci, suggerisce che la preferenza degli adulti per questa pianta sia una risposta adattativa alle condizioni di inondazione incerte dell'habitat tipico di *L. macrostigma*.

I risultati di questi studi, che riguardano le fasi chiave del ciclo vitale di *L. macrostigma*, forniscono una base scientifica allo sviluppo di azioni di conservazione mirate non solo alla specie, ma anche al ripristino degli stagni salmastri temporanei del Mediterraneo quando si utilizza *L. macrostigma* come specie bandiera. Garantire un periodo di presenza d'acqua da novembre a giugno è fondamentale per ridurre i rischi di disseccamento delle uova e per favorire le larve di *L. macrostigma* rispetto a quelle di altre specie di odonati nell'area di studio. Ciò potrebbe richiedere un'ingegneria ecologica a scala di bacino, basata sulla modellazione dell'idrologia dello stagno utilizzando dati meteorologici e topografici. Considerando i cambiamenti climatici e l'aumento dell'imprevedibilità delle precipitazioni nell'area mediterranea, l'irrigazione artificiale di alcuni stagni potrebbe garantire la sostenibilità di alcuni siti permanenti per la riproduzione. L'irrigazione può anche aiutare a controllare i livelli di salinità e a mantenere alti i livelli d'acqua durante il periodo di inondazione, per aumentare la fitness della specie. La colonizzazione di stagni di nuova creazione da parte di *B. maritimus* dovrebbe essere accelerata trapiantando stoloni e tuberi raccolti in loco. La copertura vegetale esistente dovrebbe essere mantenuta attuando uno schema di pascolo adattativo da parte del bestiame legato al regime idrico target. Considerando il degrado degli stagni salmastri temporanei del passato, una rete di questi habitat dovrebbe essere ripristinata o ricreata considerando *L. macrostigma* come specie bandiera. Il loro monitoraggio a lungo termine sarebbe utile per identificare le cause per eventuali successi o fallimenti del progetto e a migliorare la pianificazione di progetti futuri. A questo proposito, sono necessarie nuove ricerche sulla reintroduzione di *L. macrostigma* in alcuni stagni ripristinati. Questo dottorato fornisce un importante caso di studio su come considerare insetti minacciati nelle strategie di conservazione della biodiversità.

Traducionne: Sönke Hardersen & Ilaria Toni (con un ringraziamento speciale a Katia Lombardini)

## Sažetak

Kukci su skupina organizama kod kojih je zabilježen najveći pad brojnosti populacija zbog njihove iznimno velike osjetljivosti na razne antropogene utjecaje. Unatoč tome, još su uvijek uvelike zanemareni u konzervacijskim zakonodavnim aktivnostima ali i znanstvenim istraživanjima. Površina močvarnih područja drastično je smanjena tijekom zadnjih nekoliko stoljeća a prijetnje ovim važnim ekosustavima i njihovoj bioraznolikosti (uključujući i bioraznolikost kukaca) u daljnjem su porastu. Vretenca su važni bioindikator konzervacijskog statusa močvarnih područja. Obnova (restauracija) staništa (uključujući i njihovo ponovno stvaranje) trenutno predstavlja jednu od prioritarnih strategija konzervacijske biologije. Ovakve konzervacijske aktivnosti provode se uz pomoć karizmatičnih organizama (tzv. *flagship species*), no da bi one bile učinkovite, potrebno je temeljito poznavanje ekoloških zahtjeva tih vrsta. Ova doktorska disertacija usredotočena je na ugroženu vrstu, *Lestes macrostigma*, jednu od karizmatičnih vrsta koje se koriste za provedbu očuvanja i restauracije povremenih boćatih lokvi. Prije provedenog istraživanja, znanje o utjecaju hidrološkog režima i saliniteta vode na jajašca i ličinke ove vrste (ključnih stadija u dinamici populacija vretenaca), bilo je ograničeno. Kako bi se pokrenule znanstveno utemeljene konzervacijske aktivnosti, ovim radom su se nastojale popuniti praznine u poznavanju biologije ove vrste. Stoga su provedena četiri istraživanja u Camargueu, u južnoj Francuskoj. Za ovo područje karakteristične su naslage soli i sredozemna klima, koji pridonose stvaranju brojnih povremenih boćatih močvarnih staništa naseljenih populacijama vrste *L. macrostigma*, koja se tamo i uspješno razmnožava.

Kako bismo unaprijedili svoje znanje o biologiji jajašaca ove vrste, proučavali smo embrionalni razvoj i fenologiju i uspjeh izlijeganja ličinki u dvije povremene boćate lokve. Rezultati su pokazali kako su jajašca položena u izdanke biljaka tijekom kasnog proljeća, ostala u ranoj fazi razvoja tijekom ljeta, jeseni i zime. To vjerojatno povećava njihovu otpornost na ekstremne temperature i ukazuje na mehanizme prilagodbe vrste *L. macrostigma* povremenim staništima, koja su obilježena neizvjesnim hidrološkim režimom. No, kada su se biljni izdanci zaledili, došlo je do smanjenog preživljavanja jajašaca, što indicira kako bi više razine vode koja se nalazi iznad izdanaka smanjile smrtnost jajašaca uzrokovanu nižim temperaturama tijekom zime. Izlijeganje ličinki bilo je sinkronizirano i odvijalo se od sredine ožujka do sredine travnja. Razlike u fenologiji izlijeganja između istraživanih lokvi najvjerojatnije su posljedica razlika u temperaturi vode.

Salinitet je jedan od glavnih okolišnih čimbenika koji utječe na sastav zajednica kukaca. Stoga smo u laboratoriju proučavali učinak razine saliniteta na stadije jajašca i ličinke. U rasponu saliniteta 2-9,5 g/L nije zabilježen značajan učinak morske soli na preživljavanje jajašaca (uspjeh izlijeganja ličinki). Suprotno tome, preživljavanje ličinki se povećalo s razinom saliniteta od 0,5 do 8 g/L, dok je ono reducirano na nulu na 16 g/L. Povećanje saliniteta produžilo je vrijeme ličinačkog razvoja do odraslog (adultnog) stadija, smanjilo stope rasta ličinki i veličinu odraslih jedinki prilikom emergencije. Izloženost salinitetu tijekom ličinačkog stadija nije imala utjecaja na mišićnu masu toraksa odraslih jedinki, letne sposobnosti, ulaganje organizma u funkcioniranje imunološkog sustava i dostupnu energiju. Međutim, potrošnja energije se povećala prilikom povećanja saliniteta, što rezultira smanjenom količinom energije koja je na raspolaganju odraslim jedinkama. Rezultati ovih istraživanja stoga ukazuju na to da je *L. macrostigma* generalist slanih staništa (temeljna niša) čija se niša pomaknula prema boćatim vodama.

Drugi čimbenici, posebno biotičke interakcije do kojih dolazi tijekom ličinačkog stadija, mogu imati posljedice na ličinke, koje se dalje prenose i na odrasle jedinke. Stoga smo proveli terensko istraživanje na 33 lokve, kako bismo istražili učinak 14 biotičkih i abiotičkih varijabli na brojnost ličinki *L. macrostigma* i veličinu odraslih jedinki prilikom emergencije. Brojnost jedinki je bila veća u manjim lokvama i onima u kojima se hidrofaza uspostavlja kasnije, u jesensko-zimskom razdoblju. Moguće je da su u tome važnu ulogu imale i predatorske ličinke vretenaca porodice Aeshnidae, koje su bile brojnije i veće u lokvama u kojima se vodena faza uspostavlja ranije. Visoka osjetljivost ličinki vrste *L. macrostigma* na prisutnost predatora mogla bi biti rezultat njihovih visokih razina aktivnosti povezanih s njihovim brzim rastom i razvojem, koji je potreban zbog isušivanja njihovih povremenih staništa. Nadalje, jedinke vrste *L. macrostigma* koje su emergirale iz lokvi sa višim razinama vode, bile su ujedno i veće, najvjerojatnije zato što niže temperature vode produljuju razdoblje ličinačkog rasta i razvoja. S druge strane, brojnost jedinki ove vrste nije bila utjecana razinom saliniteta vode kao ni dostupnošću biljaka u kojima ženke mogu polagati jajašca.

Kako bismo istražili ima li odabir biljaka u koje odrasle jedinke (ženke) polažu jajašca utjecaj na njihovo potomstvo, koristili smo polukontrolirane uvjete kojima smo proučavali učinak uspostave hidrofaze i vrste biljnih izdanaka u koje su bila položena jajašca na uspjeh izlijeganja ličinki. Utvrdili smo da je vlaženje (uspostava vodene faze ili hidroperioda) obavezno za izlijeganje ličinki. Jajašca su preživljavala fazu presušivanja sve do uspostave vodene faze u travnju (tj. nakon provedenih 10 mjeseci u suhim uvjetima), ali uz nisku stopu preživljavanja. No kasnija uspostava hidrofaze nije rezultirala izlijeganjem ličinki. Polaganje jajašaca u ševariku (*Bolboschoenus maritimus*) omogućilo je veću stopu preživljavanja i brži embrionalni razvoj ličinki. S obzirom na to da vrsta *B. maritimus* raste u dubljim dijelovima lokvi, pa je stoga izložena ranijoj pojavi vode prilikom uspostave hidrofaze, ovi rezultati

ukazuju na to kako je sklonost odraslih jedinki vrste *L. macrostigma* ovoj biljci adaptivni odgovor na neizvjesne hidrološke uvjete tipičnog (povremenog) staništa ove vrste vretenaca.

Rezultati provedenih istraživanja, koja integriraju ključne faze životnog ciklusa *L. macrostigma*, pružaju znanstvenu osnovu za razvoj mjera očuvanja usmjerenih ne samo na ovu karizmatičnu vrstu, već ujedno i na obnovu (restauraciju) mediteranskih povremenih boćatih lokvi. Uspostava hidroperioda u razdoblju od studenog do lipnja ključna je za smanjenje rizika od isušivanja jajašaca i omogućava veće preživljavanje ličinki u odnosu na druge vrste vretenaca u području istraživanja. Kako bi se to postiglo, mogu se koristiti metode ekološkog inženjeringa na razini porječja, temeljene na modeliranju hidrologije lokvi korištenjem meteoroloških i topografskih podataka. S obzirom na klimatske promjene i sve veću nepredvidivost količine i rasporeda oborina u mediteranskom području, umjetno natapanje nekih lokvi navodnjavanjem može jamčiti održivost brojnih staništa sa stalno prisutnom vodom, na kojima se *L. macrostigma* može razmnožavati. Navodnjavanje također može pomoći u kontroli razine saliniteta i održavanju visoke razine vode tijekom hidroperioda kako bi se povećao fitnes ove vrste.

Naseljavanje novostvorenih lokvi s vrstom *B. maritimus* može se ubrzati *in situ* presađivanjem sakupljenih gomolja i izdanaka. Postojeći vegetacijski pokrov potrebno je održavati primjenom prilagodljivog sustava ispaše stokom vezanog uz ciljni hidrološki režim. Uzimajući u obzir postojeću narušenost stanja povremenih boćatih lokvi, potrebno je obnoviti ili ponovno stvoriti mrežu ovih staništa koristeći vrstu *L. macrostigma* kao karizmatičnu vrstu. Dugoročno praćenje ovih staništa doprinijelo bi razumijevanju razloga uspjeha ili neuspjeha projekta te bi poboljšalo dizajn budućih projekata. S obzirom na to, potrebna su nova istraživanja o ponovnom uvođenju vrste *L. macrostigma* u neke restaurirane lokve. Na temelju provedenih istraživanja, ova doktorska disertacija pokazuje nam na koji način uzeti u obzir ugrožene vrste u provođenju strategija očuvanja bioraznolikosti.

Prijevod: Marina Vilenica

## Összefoglalás

Jelenleg az ízeltlábúak tekinthetők a leggyorsabban hanyatló fajcsoportnak, és az emberi tevékenység által leginkább veszélyeztetettnek, mégis a természetvédelmi rendelkezések és kutatások nagymértékben figyelmen kívül hagyják őket. A vizes élőhelyek számottevően csökkentek az elmúlt évszázadokban, és továbbra is jelentős fenyegetésekkel szembesülnek, amelyek a biodiverzitás összes ágát érintik, beleértve az ízeltlábúakat is. Az Odonata-fajok (kis- és nagyszitakötők) a vizes élőhelyek természetvédelmi állapotának értékes jelzői. Az élőhelyek helyreállítása (beleértve az élőhelyek felújítását is) a jelen évtizedben elsődleges stratégiává vált. Az ilyen természetvédelmi tevékenységeket a zászlóshajófajok figyelembe vétele elősegíti, azonban a hatékonyság érdekében részletes ismereteket szükséges gyűjteni e fajok ökológiai igényeiről is. Ez a doktori értekezés a veszélyeztetett *Lestes macrostigma* kasszikötőre, mint zászlóshajófajra összpontosított, annak érdekében, hogy megvalósuljon az időszakos brakvízű kistavak természetvédelme és helyreállítása. Korlátozott tudás állt rendelkezésre a vízforgalomnak és a víz sótartalomszintjének hatásáról a tojásokra és a lárvákra, amelyek kulcsfontosságú állapotok a szitakötők populációdinamikájában. A bizonyítékokon alapuló természetvédelmi eljárások kivitelezése érdekében célunk az volt, hogy kitöltsük e hiányosságokat, így négy esettanulmányt végeztünk a dél-franciaországi Camargue-ban. Ezt a területet sólerakódások és mediterrán éghajlat jellemzi, brakvízű és időszakos vizes élőhelyeket teremtve, amelyekben a *L. macrostigma* populációi találhatóak.

A tojásbiológiával kapcsolatos ismereteink bővítése érdekében, két időszakos brakvízű kistóban tanulmányoztuk az embriófejlődést, a kikelés fenológiáját és annak sikerességét. Megállapítottuk, hogy a tojások – melyeket növényi hajtásokba raknak késő tavasszal –, korai fejlődési szakaszban maradtak a nyár, az ősz és a tél folyamán is. Ez valószínűleg növeli ellenálló képességüket az extrém hőmérsékletekkel szemben, és illusztrálja a *L. macrostigma* alkalmazkodóképességét az időszakos élőhelyekhez, amelyeknél bizonytalan az elöntés ideje. A növényi hajtások jéggel való borítottsága azonban csökkenti a tojások túlélési esélyeit, ami arra utal, hogy a hajtások feletti magasabb vízállás csökkenti a tojásoknál az alacsony téli hőmérséklet által okozott halálozási arányt. A kikelés idejének a kistavak közötti eltérése valószínűleg a vízhőmérséklet különbségeiből ered.

A víz sótartalma meghatározó tényező az ízeltlábú-közösségek szempontjából, ezért laboratóriumban tanulmányoztuk a tojás- és a lárvállapot alatti sótartalomszint hatását. A tojás



túlélésére (kikelési siker) a 2–9,5 g/L közötti tengerisó-tartalom nincs hatással. A lárvák túlélési esélye ellenben a sótartalommal együtt nőtt 0,5-től 8 g/L-ig, de nullára csökkent 16 g/L-nél. A sótartalom növekedésével az imágóállapotig nőtt a fejlődési idő, csökkent a lárvák növekedési üteme, és csökkent az átváltozáskor az imágó mérete. A lárváállapot során tapasztalt sótartalomszintnek nem volt hatása az imágó torizomtömegére, repülési teljesítményére, az immunfunkciókra és a hozzáférhető energiára sem. Az energiafogyasztás azonban nőtt a nagyobb sótartalmi szintekkel, ami alacsonyabb nettó energiamennyiséghez vezetett az imágóállapotban. Eredményeink tehát arra utalnak, hogy a *L. macrostigma* egy sógeneralista (fundamentális niche) faj, amelynek a megvalósult niche-e eltolódott a brakvizek felé.

Egyéb tényezők, különösen a biotikus interakciók, amelyekkel a lárváállapotban szembesülnek, következményekkel járhatnak a lárvákra, és befolyásolhatják az imágókat is. Ezért egy 33 tóra kiterjedő terepi tanulmányt végeztünk, 14 biotikus és abiotikus változó hatását vizsgálva a *L. macrostigma* lárváinak gyakoriságára és az imágók átváltozási méretére. Megállapítottuk, hogy a lárvák gyakoribbak voltak az apróbb kistavakban, és azokban, ahol későbbre tolódott az elöntés ideje az ősztől télig tartó időszakban. Úgy tűnt, hogy ezt a ragadozó karcsúacsa (*Aeshnidae*) szitakötőlárvák befolyásolják, amelyek gyakoribbak és nagyobbak voltak a korábbi elöntéssel érintett tavakban. A *L. macrostigma* lárváinak nagyfokú érzékenysége a ragadozókra összefügghet nagy aktivitási szintjükkel, amelyet az időszakos élőhelyeik kiszáradása által előidézett gyors növekedési és fejlődési ütemük szab meg. A *L. macrostigma* átváltozási mérete általában nagyobb azokban a kistavakban, ahol magasabb volt a vízállás, valószínűleg az alacsonyabb vízhőmérséklet miatt, ami meghosszabbítja a lárvák növekedési idejét. A víz sótartalomszintje és a tojásrakásra alkalmas növények jelenléte ellenben nem volt hatással a *L. macrostigma* lárváinak gyakoriságára.

Annak érdekében, hogy kiderítsük, vajon az imágók bizonyos tojásrakásra alkalmas növények iránt mutatott preferenciája előnyös-e az utódok számára, szemikontrollált körülmények között tanulmányoztuk az átváltozás sikerességét az elöntés dátuma és a tojásrakásra alkalmas növény típusa szerint. Arra a következtetésre jutottunk, hogy az elöntés elengedhetetlen volt az átváltozáshoz. A tojások a legkésőbb áprilisban történő elöntésig is képesek voltak túlélni (azaz 10 hónapig elöntés nélküli körülmények között voltak) – bár a túlélési arányuk ilyenkor alacsonyabb volt -, de ennél későbbi elöntés esetén már nem volt észlelhető átváltozás. Ha az imágók a tojásokat a zsiókába (*Bolboschoenus maritimus*) tojták, akkor a túlélési arány nagyobb és az embriófejlődés gyorsabb volt. Mivel a *B. maritimus* a meder mélyebb részeiben növekszik, s így korábbi elöntésnek van kitéve, ez azt sugallja, hogy az imágók preferenciája ehhez a növényhez a *L. macrostigma* esetében a jellemző élőhely bizonytalan elöntési feltételeire adott adaptív válasznak tekinthető.

Ezeknek a tanulmányoknak az eredményei, amelyek integrálják a *L. macrostigma* életciklusának kulcsfontosságú szakaszait, tudományos alapot nyújtanak olyan védelmi intézkedések kidolgozásához, amelyek nem csak a faj előfordulására, de a mediterrán időszakos brakvízű kistavak helyreállítására is irányulnak, a *L. macrostigma* kiemelt zászlóshajófajként történő használatával. A novembertől júniusig tartó hidrológiai periódus beállítása létfontosságú a tojás kiszáradási kockázatának csökkentéséhez, és a lárváknak is kedvez a többi szitakötőfajjal szemben a tanulmányozott területen belül. Ez esetben ökomérnöki megoldásokra lehet szükség vízgyűjtőléptékben, ami a meteorológiai és a topográfiai adatok felhasználásával kistóhidrológiai modellezésen alapul. Tekintettel a klímaváltozásra és a mediterrán térség csapadékmennyiségének kiszámíthatatlanságára, az öntözés révén néhány kistavat mesterségesen elárasztva garantálható lenne számos perennális szaporodóhely fenntarthatósága. Az öntözés a sótartalom szintjének beállításában és az elöntési időszak alatt a magas vízszint megtartásában is segíthet, ezzel növelve a faj túlélési esélyeit. Az újonnan létrehozott kistavaknak a *B. maritimus* általi kolonizációját in situ gyűjtött gumók és hajtások átültetésével kellene felgyorsítani. A meglévő vegetációborítást fenn kell tartani egy alkalmazkodásra képes legeltetési rendszer megvalósításával, olyan fajokat használva, amelyek kötődnek a megcélzott vízforgalomhoz. Tekintettel az időszakos brakvízű kistavak múltbeli degradációjára, az ilyen élőhelyek hálózatának helyreállításához vagy felújításához szükséges a *L. macrostigma* egyik zászlóshajófajként történő használata. A hosszú távú monitorozás segíthet a projekt esetleges sikerének vagy kudarcának okait feltárni, és javítani a jövőbeli projektek tervezését. Ebből a szempontból új kutatásokra van szükség a *L. macrostigma* visszatelepítésével kapcsolatban néhány helyreállított kistóban. Ez a doktori munka fontos esettanulmány arról, hogyan lehet figyelembe venni a veszélyeztetett rovarokat a biodiverzitás-megőrzési stratégiákban.

Fordítás: Attila Kalmár & György Dévai

## Резюме

Насекомые Насекомите са най-бързо намаляващата група видове и сред най-заstraшените от антропогенни дейности. Въпреки това, те са до голяма степен пренебрегнати в политиките за опазване на околната среда и консервационните изследвания. Влажните зони са намалели значително през последните векове и продължават да са изправени пред сериозни заплахи, засягащи всички компоненти на биоразнообразието, включително насекомите. Водните кончета (разред Odonata) са ценни индикатори за природозащитното състояние на влажните зони. Възстановяването на местообитанията е издигнато като приоритетна стратегия през настоящото десетилетие. Такива консервационни дейности се подпомагат с помощта на „флагови“ приоритетни видове. За да бъдат консервационните дейности ефективни, е необходимо задълбочено познаване на екологичните изисквания на флаговите видове. Тази докторска дисертация се фокусира върху застрашения вид водно конче тъмно смарагдово водно шило *Lestes macrostigma*, като флагов вид при опазването и възстановяването на временни бракични водоеми. Досега беше налична ограничена информация относно влиянието на хидрологичния режима и солеността на водата върху яйцата и ларвите, които са ключови етапи в динамиката на популациите при водните кончета. С целпредприемане на научнообосновани консервационни дейности, ние си поставихме за цел да запълним тези пропуски в знанията, като проведохме четири проучвания в Камарг, Южна Франция. Тази област се характеризира със солени отлагания и средиземноморски климат, създаващи бракични и временни водоеми, които са местообитания за размножаващи се популации на *L. macrostigma*.

Първо, с цел подобряване на знанията за биологията на яйцата, ние проучихме фенологията на ембрионалното развитие и люпилния успех в две временни бракични езера. Установихме, че яйцата, снесени в стъблата на растенията през късната пролет остават в ранен стадий на развитие през лятото, есента и зимата. Това вероятно увеличава тяхната устойчивост на екстремни температури и показва адаптирането на *L. macrostigma* към временни местообитания с несигурен период на оводняване. Замръзването на водата около растенията намалява оцеляването на яйцата, което предполага, че водни нива по-високи от стъблата на растенията биха намалили смъртността на яйцата, причинена от по-ниските зимни температури. Периодът на развитие на яйцата продължи от средата на март до средата на април и

изключването беше синхронизирано. Разликите във фенологията на излюпване сред езерата най-вероятно се дължат на разликите в температурите на водата.

Солеността на водата е основен фактор за съобществата от насекоми. Ето защо проучихме в лабораторни условия ефекта от нивата на соленост, наблюдавани по време на етапите на развитие на яйцата и ларвите. Не открихме ефект от солеността на водата върху оцеляването на яйцата (успех на излюпване) в концентрации от диапазона 2–9,5 g/L. Обратното беше установено за ниво на соленост от 0,5 до 8 g/L – преживяемостта на ларвите се увеличава, но намалява до нула при 16 g/L. Повишаването на нивата на соленост увеличава времето за развитие до стадий на имаго, намалява темповете на растеж на ларвите и намалява размера на възрастните при метаморфозирание. Нивото на соленост, наблюдавано в стадия на ларвата, нямаше ефект върху мускулната маса на торакса при имагото, полетните характеристики, инвестициите в имунната функция и наличната енергия. Въпреки това, консумацията на енергия се увеличава с по-високи нива на соленост, което води до по-нисък нетен енергиен бюджет в стадия на имаго. Нашите резултати следователно показват, че *L. macrostigma* е солен генералист. Това е фундаменталната ниша, от която реализираната ниша се измества към бракични води.

Други фактори, особено биотични взаимодействия, изпитани по време на ларвния стадий, могат да имат последствия върху ларвите и да засегнат възрастните. Затова проведохме теренно проучване в 33 водоема, тествайки ефекта на 14 биотични и абиотични фактора върху обилието на ларвите и размера на възрастните на *L. macrostigma*. Установихме, че ларвите са по-обилни в по-малки езера и с по-късно оводняване през есента и зимата. Това изглежда се дължи на хищни ларви на други видове водни кончета, които са по-изобилни и по-големи в по-рано наводнените водоеми. Високата уязвимост на ларвите на *L. macrostigma* към хищници може да бъде отдадена на техните високи нива на активност, свързани с бързия им растеж и скорост на развитие, наложени от изсъхването на техните временни местообитания. Освен това *L. macrostigma* има тенденция да излиза с по-големи размери от езера с по-високи водни нива, най-вероятно защото по-ниските температури на водата удължават периода на растеж на ларвите. За разлика от това, ние не открихме никакви ефекти от нивата на соленост на водата и наличието на растения за отлагане на яйца върху изобилието на ларвите на *L. macrostigma*.

За да проучим дали предпочитанията на възрастните към снасяне на яйца върху определени растения са от полза за потомството, използвахме полуконтролирани условия, за да проучим ефекта върху успеха на излюпването според датата на наводняване и вида на растението, където са били снесени яйцата. Установихме, че намокрянето е задължително за излюпването. Яйцата успяха да се справят с изсъхването до оводняване чак през април (т.е. след

10 месеца в ненаводнени условия), но с ниска степен на оцеляване – но по-късното заливане не доведе до излюпване. Снасянето на яйца в морския болбошонус *Bolboschoenus maritimus* осигурява по-висок процент на оцеляване и по-бързо ембрионално развитие. Като се има предвид, че *B. maritimus* расте в по-дълбоките части на водоемите, като по този начин претърпява по-ранно оводняване, предполагаме, че предпочитанието на възрастните към това растение е адаптивна реакция към несигурните условия на наводняване на типичното местообитание на *L. macrostigma*.

Резултатите от тези проучвания, които интегрират ключови етапи от жизнения цикъл на *L. macrostigma*, предоставят научна основа за разработване на консервационни дейности, насочени не само към вида, но и към възстановяването на средиземноморските временни бракични езера, когато се използва *L. macrostigma* като флагов вид. Управлението на водните нива в периода от ноември до юни е от решаващо значение за намаляване на рисковете от изсушаване на яйцата и за създаване на благоприятни условия за ларвите им пред тези на други видове водни кончета в района на изследване. Това може да изисква екологично инженерство на ниво водосбор, базирано на моделиране на хидрологията на водоема с помощта на метеорологични и топографски данни. Предвид изменението на климата и увеличаването на непредсказуемостта на валежите в средиземноморската зона, наводняването на някои езера изкуствено чрез напояване може да гарантира устойчивостта на редица дългогодишни места за възпроизводство на вида. Напояването може също така да помогне за контролиране на нивата на соленост и поддържане на високи нива на водата по време на периода на наводнение, за да се подобрят условията за вида. Колонизирането на новосъздадени водоеми от *B. maritimus* трябва да се ускори чрез засаждане на материал, събран *in situ*. Съществуващата растителна покривка трябва да се поддържа чрез прилагане на адаптивна схема за паша от добитък, свързана с целевия хидрологичен режим. Като се има предвид намалението на временните бракични водоеми в миналото, мрежа от тези местообитания трябва да бъде възстановена или пресъздадена с помощта на *L. macrostigma* като флагов вид. Техният дългосрочен мониторинг би помогнал да се идентифицират причините за успеха или провала на проекта и да се подобри дизайнът на бъдещи проекти. В тази връзка са необходими нови изследвания за повторното въвеждане на *L. macrostigma* в някои възстановени езера. Тази докторска дисертация предоставя важен казус за това как да се вземат предвид застрашените насекоми в рамките на стратегиите за опазване на биоразнообразието.

Превод: Станимира Делева и Димитър Попов (Stanimira Deleva & Dimitar Popov)

## Περίληψη

Τα έντομα είναι η ταχύτερα μειούμενη, ομάδα ειδών και ανάμεσα στις πλέον απειλούμενες από τις ανθρωπογενείς δραστηριότητες. Ωστόσο, στις πολιτικές διατήρησης και στην έρευνα για τη διατήρηση παραβλέπονται σε μεγάλο βαθμό. Κατά τους τελευταίους αιώνες οι υγρότοποι έχουν μειωθεί σημαντικά και συνεχίζουν να αντιμετωπίζουν σημαντικές απειλές που επηρεάζουν πληθώρα οργανισμών, συμπεριλαμβανομένων των εντόμων. Τα Οδοντόγναθα (λιβελούλες) αποτελούν πολύτιμους βιοδείκτες της κατάστασης διατήρησης των υγροτόπων. Η αποκατάσταση ενδιαιτημάτων (συμπεριλαμβανομένου του επανασχεδιασμού ενδιαιτημάτων) έχει αναδειχθεί ως στρατηγική προτεραιότητας κατά την τρέχουσα δεκαετία. Τέτοιου είδους δράσεις διατήρησης διευκολύνονται με τη χρήση εμβληματικών ειδών αλλά για να είναι αποτελεσματικές, απαιτείται ενδελεχής γνώση των οικολογικών τους απαιτήσεων. Η παρούσα διδακτορική διατριβή επικεντρώθηκε στην απειλούμενη λιβελούλα *Lestes macrostigma*, ως εμβληματικό είδος για την υλοποίηση δράσεων διατήρησης και αποκατάστασης εποχιακών υφάλμυρων λιμνίων. Οι γνώσεις μας σχετικά με την επίδραση του υδρολογικού καθεστώτος και των επιπέδων αλατότητας του νερού στα ωά και τις προνύμφες του είδους, τα οποία αποτελούν βασικά στάδια στη δυναμική των πληθυσμών των Οδοντόγναθων, είναι περιορισμένες. Με στόχο την ανάληψη δράσεων διατήρησης βασισμένων σε τεκμήρια, επιδιώξαμε να καλύψουμε αυτά τα κενά γνώσης με τη διεξαγωγή τεσσάρων μελετών στην Camargue της νότιας Γαλλίας. Η περιοχή αυτή χαρακτηρίζεται από αποθέσεις αλάτων και μεσογειακό κλίμα, δημιουργώντας υφάλμυρα και εποχιακά συστήματα εσωτερικών υδάτων που φιλοξενούν αναπαραγωγικούς πληθυσμούς του *L. macrostigma*.

Για να βελτιώσουμε τις γνώσεις μας σχετικά με τη βιολογία των ωών, μελετήσαμε την εμβρυϊκή ανάπτυξη καθώς και τη φαινολογία και επιτυχία εκκόλαψης σε δύο εποχιακά υφάλμυρα λιμνία. Διαπιστώσαμε ότι τα αυγά – τα οποία εναποτίθενται σε βλαστούς φυτών κατά τα τέλη της άνοιξης – παρέμειναν σε πρώιμο στάδιο ανάπτυξης κατά τη διάρκεια του καλοκαιριού, του φθινοπώρου και του χειμώνα. Αυτό πιθανότατα αυξάνει την ανθεκτικότητά τους σε ακραίες θερμοκρασίες καταδεικνύοντας την προσαρμογή του *L. macrostigma* σε εποχιακά ενδιαιτήματα όπου τα πλημμυρικά φαινόμενα δεν παρουσιάζουν σταθερή περιοδικότητα. Ωστόσο, η εναπόθεση ωών σε βλαστούς φυτών που παγώνουν κατά τη χειμερινή περίοδο μειώνει την βιωσιμότητά τους, γεγονός που υποδηλώνει ότι επίπεδα νερού υψηλότερα από το ύψος των βλαστών θα μείωναν τη

θνησιμότητα των ωών που προκαλείται από τις χαμηλότερες θερμοκρασίες του χειμώνα. Η εκκόλαψη διήρκεσε από τα μέσα Μαρτίου έως τα μέσα Απριλίου και ήταν συγχρονισμένη. Οι διαφορές στη φαινολογία εκκόλαψης μεταξύ των λιμνίων οφείλονταν πιθανότατα σε διαφορές στις θερμοκρασίες του νερού.

Η αλατότητα του νερού είναι ένας σημαντικός παράγοντας που επηρεάζει τις βιοκοινότητες εντόμων. Ως εκ τούτου, μελετήσαμε στο εργαστήριο την επίδραση των επιπέδων αλατότητας που παρατηρούνται κατά τη διάρκεια των σταδίων των ωών και των προνυμφών. Δεν διαπιστώσαμε καμία επίδραση της αλατότητας στην επιβίωση των ωών (επιτυχία εκκόλαψης) στο εύρος 2-9.5 g/L. Αντίθετα, η επιβίωση των προνυμφών αυξήθηκε σε επίπεδα αλατότητας από 0.5 έως 8 g/L, αλλά μηδενίστηκε στα 16 g/L. Η αύξηση των επιπέδων αλατότητας αύξησε το χρόνο ανάπτυξης μέχρι το στάδιο του ενήλικου εντόμου, μείωσε τους ρυθμούς ανάπτυξης των προνυμφών και μείωσε το μέγεθος των ενηλίκων ατόμων κατά τη μεταμόρφωσή τους. Το επίπεδο αλατότητας κατά το στάδιο της προνύμφης δεν είχε καμία επίδραση στη θωρακική μυϊκή μάζα των ενηλίκων ατόμων, στις επιδόσεις πτήσης, στην ανοσολογική λειτουργία και στη διαθέσιμη ενέργεια τους. Ωστόσο, η κατανάλωση ενέργειας αυξήθηκε σε υψηλότερα επίπεδα αλατότητας, έχοντας ως αποτέλεσμα ένα χαμηλότερο καθαρό προϋπολογισμό ενέργειας στο στάδιο του ενήλικου. Συνεπώς, τα αποτελέσματά μας έδειξαν ότι το *L. macrostigma* είναι ένα ευρύοικο - ως προς την αλατότητα - είδος (θεμελιώδης οικοθέση) του οποίου η οικοθέση μετατοπίστηκε προς τα υφάλμυρα νερά.

Άλλοι παράγοντες, ιδίως οι βιοτικές αλληλεπιδράσεις, που σημειώνονται κατά τη διάρκεια του προνυμφικού σταδίου μπορεί να έχουν συνέπειες στις προνύμφες επηρεάζοντας τα ενήλικα άτομα. Ως εκ τούτου, πραγματοποιήσαμε μια μελέτη πεδίου σε 33 λιμνία, εξετάζοντας την επίδραση 14 βιοτικών και αβιοτικών μεταβλητών στην αφθονία των προνυμφών του *L. macrostigma* και στο μέγεθος των ενηλίκων κατά τη μεταμόρφωσή τους. Διαπιστώσαμε ότι οι προνύμφες ήταν πιο άφθονες σε μικρότερα λιμνία και με μεταγενέστερα πλημμυρικά φαινόμενα κατά το φθινόπωρο-χειμώνα. Αυτό φάνηκε να διαμεσολαβείται από προνύμφες αρπακτικών λιβελούλων της οικογένειας Aeshnidae, οι οποίες ήταν πιο άφθονες και μεγαλύτερες σε λιμνία που πλημμύριζαν νωρίτερα. Η υψηλή ευαισθησία των προνυμφών του *L. macrostigma* σε θηρευτές μπορεί να σχετίζεται με την έντονη δραστηριότητά τους, που συνδέονται με τον γρήγορο ρυθμό αύξησης και ανάπτυξής τους, ο οποίος επιβάλλεται από την αποξήρανση των προσωρινών ενδιαιτημάτων τους. Επιπλέον, τα ενήλικα άτομα *L. macrostigma* έτειναν να έχουν μεγαλύτερο μέγεθος στα λιμνία με υψηλότερη στάθμη νερού, πιθανότατα επειδή οι χαμηλότερες θερμοκρασίες νερού παρατείνουν την περίοδο ανάπτυξης των προνυμφών. Αντίθετα, δεν διαπιστώσαμε καμία επίδραση των επιπέδων αλατότητας του νερού και της διαθεσιμότητας φυτών για εναπόθεση των ωών στην αφθονία των προνυμφών του *L. macrostigma*.

Προκειμένου να διερευνήσουμε κατά πόσον η προτίμηση των ενηλίκων ατόμων σε ορισμένα φυτά για την εναπόθεση των ωών τους ωφελεί τους απογόνους τους, χρησιμοποιήσαμε ήμι-ελεγχόμενες συνθήκες για να μελετήσουμε την επίδραση στην επιτυχία της εκκόλαψης ανάλογα με την ημερομηνία πλημμυρίσματος και τον τύπο βλαστού του φυτού όπου εναποτέθηκαν τα ωά. Διαπιστώσαμε ότι η κατάβρεξη ήταν υποχρεωτική για την εκκόλαψη. Τα ωά ήταν σε θέση να ανταπεξέλθουν στην περίοδο αποξήρανσης μέχρι το πλημμύρισμα των λιμνίων έως και τον Απρίλιο (δηλαδή μετά από 10 μήνες σε μη πλημμυρισμένες συνθήκες) - ακόμη και με χαμηλό ποσοστό επιβίωσης - αλλά πλημμυρικά φαινόμενα μετά το πέρας της περιόδου αυτής είχαν ως αποτέλεσμα να μην πραγματοποιηθεί εκκόλαψη. Η εναπόθεση ωών στο φυτό *Bolboschoenus maritimus* παρείχε υψηλότερο ποσοστό επιβίωσης και ταχύτερη εμβρυϊκή ανάπτυξη. Δεδομένου ότι το *B. maritimus* αναπτύσσεται σε βαθύτερα τμήματα του λιμνίου, με αποτέλεσμα να υφίσταται νωρίτερα τις πλημμύρες, υποδηλώνει ότι η προτίμηση των ενηλίκων για το φυτό αυτό αποτελεί προσαρμοστική απάντηση στα τυπικά ενδιαιτήματα του *L. macrostigma* όπου πλημμυρικά φαινόμενα δεν παρουσιάζουν σταθερή περιοδικότητα.

Τα αποτελέσματα των μελετών αυτών, οι οποίες ενσωμάτωσαν βασικά στάδια του κύκλου ζωής του *L. macrostigma*, παρέχουν μια επιστημονική βάση για την ανάπτυξη δράσεων διατήρησης με στόχο όχι μόνο το είδος, αλλά και την αποκατάσταση των μεσογειακών εποχικών υφάλμυρων λιμνίων, όταν το *L. macrostigma* χρησιμοποιείται ως εμβληματικό είδος. Ο καθορισμός μιας υδροπεριόδου από τον Νοέμβριο έως τον Ιούνιο είναι ζωτικής σημασίας για τη μείωση των κινδύνων αποξήρανσης των ωών και για την ευνοϊκή μεταχείριση των προνυμφών του έναντι των προνυμφών άλλων ειδών Οδοντόγναθων στην περιοχή μελέτης. Αυτό μπορεί να απαιτεί οικολογική μηχανική στο επίπεδο της λεκάνης απορροής, βάσει μοντελοποίησης της υδρολογίας του λιμνίου με τη χρήση μετεωρολογικών και τοπογραφικών δεδομένων. Δεδομένης της κλιματικής αλλαγής και της αύξησης του απρόβλεπτου των βροχοπτώσεων στην περιοχή της Μεσογείου, ο τεχνητός πλημμυρισμός ορισμένων λιμνίων με άρδευση μπορεί να εγγυηθεί τη βιωσιμότητα ενός αριθμού τόπων αναπαραγωγής πολυετούς διάρκειας. Η άρδευση μπορεί επίσης να βοηθήσει στον έλεγχο των επιπέδων αλατότητας και στη διατήρηση υψηλών επιπέδων νερού κατά την περίοδο πλημμύρας, προκειμένου να αυξηθεί η αρμοστικότητα των ειδών. Ο αποικισμός νέο-δημιουργηθέντων λιμνίων από το *B. maritimus* θα πρέπει να επιταχυνθεί με τη μεταφύτευση κονδύλων και παραφυάδων που συλλέγονται επί τόπου. Η υπάρχουσα φυτοκάλυψη θα πρέπει να διατηρηθεί με την εφαρμογή ενός προσαρμοστικού συστήματος βόσκησης από ζώα που συνδέονται με το υδρολογικό καθεστώς-στόχο. Λαμβάνοντας υπόψη την προηγούμενη υποβάθμιση των εποχιακών υφάλμυρων λιμνίων, ένα δίκτυο αυτών των ενδιαιτημάτων θα πρέπει να αποκατασταθεί ή να αναδημιουργηθεί χρησιμοποιώντας το *L. macrostigma* ως εμβληματικό είδος. Η μακροχρόνια παρακολούθησή τους θα βοηθούσε στον



εντοπισμό των λόγων επιτυχίας ή αποτυχίας του έργου και θα βελτίωνε τον σχεδιασμό μελλοντικών έργων. Από την άποψη αυτή, απαιτείται περεταίρω έρευνα σχετικά με την επανεισαγωγή του *L. macrostigma* σε ορισμένες αποκαταστημένες λίμνες. Η διδακτορική αυτή διατριβή παρέχει μια σημαντική μελέτη περίπτωσης σχετικά με το πώς να λαμβάνονται υπόψη απειλούμενα έντομα στο πλαίσιο στρατηγικών διατήρησης βιοποικιλότητας.

Μετάφραση : Ιάκωβος Δημητρίου

## Özet

Böcekler en hızlı azalan tür grubudur ve insan kaynaklı faaliyetler tarafından en çok tehdit altındaki türler arasındadır. Ancak, koruma politikalarında ve koruma araştırmalarında büyük ölçüde göz ardı edilmektedirler. Sulak alanlar son yüzyılda büyük ölçüde azalmıştır ve böcekler de dahil olmak üzere biyolojik çeşitliliğin tüm sektörlerini etkileyen önemli tehditlerle karşı karşıya kalmaya devam etmektedir. Odonatlar (kızböcekleri ve yusufçuklar) sulak alanların koruma durumunun değerli göstergeleridir. Habitat restorasyonu (habitat rekreasyonu dahil) içinde bulunduğumuz on yıl boyunca öncelikli bir strateji olarak gündeme gelmiştir. Bu tür koruma eylemleri amiral gemisi türler kullanılarak kolaylaştırılır, ancak etkili olabilmeleri için ekolojik gereksinimlerinin kapsamlı bir şekilde bilinmesi gerekir. Bu doktora tezi, geçici acı su göletlerinin korunması ve restorasyonunu uygulamak için bir amiral gemisi tür olarak tehdit altındaki kızböceği *Lestes macrostigma*'ya odaklanmıştır. Odonat popülasyon dinamiklerinde kilit aşamalar olan yumurta ve nimfler üzerinde su rejimlerinin ve su tuzluluk seviyelerinin etkisine ilişkin sınırlı bilgi vardır. Kanıta dayalı koruma eylemleri gerçekleştirmek amacıyla, Güney Fransa'daki Camargue'da dört çalışma yürüterek bu bilgi boşluklarını doldurmayı amaçladık. Bu alan, *L. macrostigma* üreme popülasyonlarını barındıran acı ve geçici sulak alan habitatları yaratan tuz yatakları ve Akdeniz iklimi ile karakterize edilmektedir.

Yumurta biyolojisi hakkındaki bilgilerimizi geliştirmek için, iki geçici acı su havuzunda embriyonik gelişim, kuluçka fenolojisi ve başarısı üzerinde çalıştık. İlkbaharın sonlarında bitki sürgünlerine bırakılan yumurtaların yaz, sonbahar ve kış boyunca erken gelişim aşamasında kaldığını tespit ettik. Bu durum muhtemelen yumurtaların aşırı sıcaklıklara karşı direncini artırmakta ve *L. macrostigma*'nın su baskını zamanlamasının belirsiz olduğu geçici habitatlara adaptasyonunu göstermektedir. Bununla birlikte, bitki sürgünlerinin buza gömülmesi yumurtaların hayatta kalmasını azaltmaktadır, bu da sürgünlerin üzerindeki daha yüksek su seviyelerinin düşük kış sıcaklıklarının neden olduğu yumurta ölümlerini azaltacağını düşündürmektedir. Kuluçka Mart ortasından Nisan ortasına kadar tamamlanmakta ve senkronize olmuştur. Havuzlar arasında kuluçka fenolojisindeki farklılıklar büyük olasılıkla su sıcaklıklarındaki farklılıklardan kaynaklanmaktadır.

Su tuzluluğu böcek toplulukları üzerinde önemli bir etkidir. Bu nedenle, laboratuvarında yumurta ve nimf aşamalarında yaşanan tuzluluk seviyelerinin etkisini inceledik. Deniz tuzunun 2-9,5 g/L aralığında yumurtaların hayatta kalması (kuluçka başarısı) üzerinde bir etkisi olmadığını tespit ettik.

Buna karşılık, nimf sağkalımı 0,5 ila 8 g/L tuzluluk seviyesiyle artmakta, ancak 16 g/L'de sıfıra düşmektedir. Artan tuzluluk seviyeleri yetişkin aşamasına kadar olan gelişim süresini artırmış, nimf büyüme oranlarını düşürmüş ve çıkıştaki yetişkin boyutunu azaltmıştır. Nimf aşamasında yaşanan tuzluluk seviyesinin yetişkin toraks kas kütlesi, uçuş performansı, bağışıklık fonksiyonuna yapılan yatırım ve mevcut enerji üzerinde hiçbir etkisi olmamıştır. Bununla birlikte, enerji tüketimi daha yüksek tuzluluk seviyeleriyle artmış, bu da yetişkin aşamasında daha düşük bir net enerji bütçesiyle sonuçlanmıştır. Bu nedenle sonuçlarımız, *L. macrostigma*'nın temel olarak tuzlu sulara uyum sağlamış [saline generalist] (temel niş) olduğunu ve gerçekleşen nişinin acı sulara doğru kaydığını göstermiştir.

Nimf evresinde deneyimlenen diğer faktörler özellikle biyotik etkileşimlerin, nimfler üzerinde sonuçları olmaktadır ve yetişkinleri etkilemeye devam edebilir. Bu nedenle, 33 gölette 14 biyotik ve abiyotik değişkenin *L. macrostigma* nimf bolluğu ve çıkıştaki yetişkinliğe erişme boyutu üzerindeki etkisini test eden bir saha çalışması yürüttük. Nimflerin daha küçük göletlerde ve sonbahar-kış aylarında daha geç su basan göletlerde daha bol olduğunu tespit ettik. Bu duruma, daha erken sular altında kalan göletlerde daha bol ve daha büyük olan avcı aeshnid yusufçuk nimflerinin aracılık ettiği (sebebi olduğu) görülmüştür. *L. macrostigma* nimflerinin avcılara karşı yüksek duyarlılığı, geçici yaşam alanlarının kurumasının getirdiği hızlı büyüme ve gelişme hızlarıyla ilişkili yüksek aktivite seviyeleriyle ilgili olabilir. Ayrıca, *L. macrostigma* daha yüksek su seviyesine sahip havuzlardan daha büyük (yetişkin formlarının) ortaya çıkma eğilimi göstermiştir, bunun nedeni büyük olasılıkla düşük su sıcaklıklarının nimflerin büyüme dönemini uzatmasıdır. Buna karşın, su tuzluluk seviyelerinin ve yumurtlama bitkilerinin mevcudiyetinin *L. macrostigma* nimf bolluğu üzerinde herhangi bir etkisi bulunmamıştır.

Erginlerin yumurtalarını bırakmak için belirli bitkileri tercih etmelerinin yavrulara fayda sağlayıp sağlamadığını araştırmak amacıyla, su basma tarihine ve yumurtaların bırakıldığı bitki sürgünü türüne göre kuluçka başarısı üzerindeki etkiyi incelemek için yarı kontrollü koşullar kullandık. Yumurtadan çıkma için ıslatmanın zorunlu olduğunu tespit ettik. Yumurtalar Nisan ayının sonlarına doğru (yani 10 ay boyunca su basmamış koşullarda) su basana kadar kuruma ile başa çıkabilmiştir – ancak hayatta kalma oranı düşüktür – ancak daha sonra su basması yumurtaların açılmaması ile sonuçlanmıştır. Yumurtaların *Bolboschoenus maritimus* deniz sazına bırakılması daha yüksek hayatta kalma oranı ve daha hızlı embriyonik gelişim sağlamıştır. *B. maritimus*'un göletin daha derin kısımlarında yetiştiği ve bu nedenle daha erken su baskınına maruz kaldığı göz önüne alındığında, yetişkinlerin bu bitkiyi tercih etmesinin, *L. macrostigma*'nın tipik yaşam alanının belirsiz su baskını koşullarına uyarlanabilir bir yanıt olduğunu düşündürmektedir.

*Lestes macrostigma*'nın yaşam döngüsünün kilit aşamalarını bütünleştiren bu çalışmaların sonuçları, sadece türü değil, aynı zamanda *L. macrostigma*'yı öncü tür olarak kullanırken Akdeniz geçici

acı su göletlerinin restorasyonunu da hedefleyen koruma eylemleri geliştirmek için bilimsel bir temel sağlamaktadır. Kasım-Haziran ayları arasında bir ıslak bir dönemin uygulanması, yumurtaların kuruma riskini azaltması ve larvalarını çalışma bölgesindeki diğer odonat türlerine kıyasla daha avantajlı hale gelmesi için çok önemlidir. Bu, meteorolojik ve topografik veriler kullanılarak gölet hidrolojisinin modellenmesine dayalı olarak havza ölçeğinde ekolojik mühendislik gerektirebilir. İklim değişikliği ve Akdeniz bölgesindeki yağışların öngörülemezliğindeki artış göz önüne alındığında, bazı göletlerin yapay olarak sulanması/su verilmesi, daimi üreme alanlarının sürdürülebilirliğini garanti edebilir. Sulama aynı zamanda tuzluluk seviyelerini kontrol etmeye ve türlerin uyumunu (formda olmasını) artırmak için taşkın döneminde yüksek su seviyelerini korumaya yardımcı olabilir. Yeni oluşturulan göletlerin *B. maritimus* tarafından kolonizasyonu, *in situ* (yerinde) toplanan yumru ve klonal gelişen sürgünler nakledilerek hızlandırılmalıdır. Mevcut bitki örtüsü, hedef su rejimine bağlı olarak çiftlik hayvanları tarafından uyarlanabilir bir otlatma planı uygulanarak korunmalıdır. Geçici acı su göletlerinin geçmişteki bozulması göz önünde bulundurularak, bu habitatlardan oluşan bir ağ, *L. macrostigma* öncü tür olarak kullanılarak restore edilmeli veya yeniden oluşturulmalıdır. Bunların uzun vadeli izlenmesi, projenin başarı veya başarısızlık nedenlerinin belirlenmesine yardımcı olacak ve gelecekteki projelerin tasarımını geliştirecektir. Bu bağlamda, restore edilen bazı göletlerde *L. macrostigma*'nın yeniden tanıtılmasıyla ilgili yeni araştırmalara ihtiyaç vardır. Bu doktora çalışması, tehdit altındaki böceklerin biyoçeşitlilik koruma stratejilerinde nasıl dikkate alınacağına dair önemli bir vaka çalışması sunmaktadır.

Çeviri: Özge Balkız & Kerim Çiçek

## Резюме

Насекомые – группа, испытывающая наиболее быстрый упадок и наиболее страдающая от деятельности человека. В то же время они в основном остаются за кадром природоохранной политики и исследований. Естественные водно-болотные угодья сильно сократились за последние столетия и продолжают сталкиваться с серьезными угрозами, влияющими на все компоненты биоразнообразия, включая стрекоз. Стрекозы являются важными индикаторами природоохранного статуса водных угодий. В текущее десятилетие приоритетной стратегией стало восстановление местообитаний (в том числе в рекреационных целях). Такого рода природоохранная деятельность облегчается использованием индикаторных видов, но для ее эффективности необходимы детальные знания их экологических требований. В фокусе данной докторской диссертации находится находящаяся под угрозой лютка *Lestes macrostigma*, являющаяся индикаторным видом для охраны и восстановления солоноватых временных водоемов. Существующие сведения относительно влияния гидрологического режима и солености воды на яйца и личинок, которые являются ключевыми стадиями в популяционной динамике стрекоз, весьма ограничены. Чтобы закрыть эти пробелы в наших знаниях в целях разработки объективно обоснованных природоохранных действий мы провели четыре исследования в Камарге, южная Франция. Этот регион характеризуется солевыми отложениями и средиземноморским климатом, что создает временные солоноватоводные местообитания, пригодные для существования популяций *L. macrostigma*.

Для пополнения наших знаний о стадии яйца мы исследовали эмбриональное развитие, фенологию и успешность выхода личинок в двух временных солоноватых водоемах. Мы обнаружили, что яйца, откладываемые в побеги растений поздней весной, пребывают на ранней стадии развития летом, осенью и зимой. Вероятно, это повышает их устойчивость к экстремальным температурам и является проявлением приспособления *L. macrostigma* к временным водоемам с неопределенным временным режимом заполнения. Однако заключение побегов растений в лед уменьшает выживаемость яиц, предполагая тем самым, что более высокий уровень воды над побегами сокращает смертность яиц от низких зимних температур. Выход личинок из яиц продолжался с середины марта по середину апреля и был

синхронным. Различия между прудами в фенологии выхода скорее всего определялись различиями температуры воды.

Соленость воды – важный фактор, влияющий на сообщества насекомых, поэтому мы исследовали эффект уровня солености на яйца и личинок в лаборатории. Мы не наблюдали влияния морской соли на выживаемость яиц (успешность выплода) в диапазоне концентраций 2–9.5 г/л. В то же время выживаемость личинок повышалась с ростом солености от 0.5 до 8 г/л, но сократилась до нуля при 16 г/л. Увеличение солености увеличивало время развития до взрослой стадии, уменьшало скорость роста личинок и уменьшало размер имаго при выплоде. Уровень солености на личиночной стадии не влиял на массу грудной мускулатуры, эффективность полета, иммунную функцию и доступную энергию. В то же время потребление энергии увеличивалось при повышении солености, что уменьшало чистый энергетический бюджет ко взрослой стадии. Таким образом, наши результаты характеризуют *L. macrostigma* как генералиста в отношении солености (фундаментальная ниша), реализованная ниша которого сдвинута к солоноватым водам.

Другие факторы, в особенности биотические взаимодействия, действующие на личиночной стадии, могут иметь последствия для личинок, переходящие и на взрослую стадию. Поэтому мы провели полевое исследование 33 водоемов на предмет влияния 14 биотических и абиотических параметров на обилие личинок *L. macrostigma* и размер имаго при выплоде. Мы выяснили, что личинки были более многочисленны в более мелких водоемах и при более позднем их затоплении осенью-зимой. Скорее всего этот эффект связан с прессом хищных личинок коромысел, которые более многочисленны в более крупных и раньше затопляемых водоемах. Высокая подверженность личинок *L. macrostigma* влиянию хищников может быть связана с их высоким уровнем активности, в свою очередь связанным с их высокой скоростью роста и развития, диктуемым быстрым высыханием их временных местообитаний. Также *L. macrostigma* имели склонность к более позднему выплоду на водоемах с более высоким уровнем воды, вероятно в связи с тем, что более низкие температуры воды удлинляли период роста личинок. Наоборот, мы не обнаружили никакого эффекта на обилие личинок *L. macrostigma* уровня солености воды и доступности подходящих для яйцекладки растений.

Чтобы исследовать приносит ли предпочтение взрослыми стрекозами определенных растений для яйцекладки какую либо пользу личинкам мы исследовали влияние даты затопления и вид растения, на которое были отложены яйца, на успешность их выплода в полуконтролируемых условиях. Мы выяснили, что затопление является обязательным условием для выплода. Яйца переносили высыхание до затопления до апреля (т. е. в течение 10 месяцев

в незатопленном состоянии) – однако с низкой выживаемостью – но затопление в более позднее время приводило к тому, что личинки уже не выходили. Откладывание яиц на клубнекамыш приморский (*Bolboschoenus maritimus*) давало более высокую выживаемость и более быстрое эмбриональное развитие. То, что *B. maritimus* растет в более глубоких частях водоемов и тем самым затопляется раньше, предполагает, что предпочтение взрослыми этого растения есть адаптивный ответ на неопределенные условия затопления в типичном местообитании *L. macrostigma*.

Результаты этих исследований, интегрирующих ключевые стадии жизненного цикла *L. macrostigma*, предоставляют научную основу для разработки природоохранных действий, направленных не только на этот вид, но также и на восстановления временных солоноватых водоемов средиземноморского типа в целом, используя *L. macrostigma* в качестве индикаторного вида. Установление определенного гидропериода с ноября по июнь является ключевым для уменьшения риска высыхания яиц и благоприятствования для личинок этого вида в противовес личинкам других стрекоз исследуемого региона. Это может потребовать экологической инженерии в масштабах водного бассейна, основанной на моделировании гидрологического режима водоемов с использованием метеорологических и топографических данных. В условиях изменения климата и увеличения непредсказуемости осадков в Средиземноморье искусственное затопление некоторых водоемов посредством ирригации могло бы гарантировать устойчивость ряда круглогодичных репродуктивных сайтов. Ирригация также может помочь контролировать уровни солености и устанавливать высокий уровень воды во время периода затопления с целью повышения приспособленности вида. Колонизация вновь создаваемых водоемов *B. maritimus* должна быть ускорена транспортировкой клубней и отводков, собираемых *in situ*. Существующий растительный покров должен поддерживаться за счет схемы адаптивного выпаса скота, связанного с целевым гидрорежимом. Учитывая деградацию солоноватых водоемов в прошлом, сеть таких водоемов должна быть восстановлена или вновь создана, с использованием *L. macrostigma* в качестве индикаторного вида. Их многолетний мониторинг помог бы установить причины успеха или неуспеха проекта и улучшить план следующих проектов. В этом аспекте необходимо новое исследование по реинтродукции *L. macrostigma* в некоторые восстановленные водоемы. Эта диссертация представляет собой исследование важного частного случая учета находящегося под угрозой насекомого в стратегии охраны биоразнообразия.

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