Predators promote trait diversification in prey

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)
submitted to the Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

by

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From Shandong, China

2018

The work in this thesis was performed during October 2014 to August 2018 under the supervision of Prof. Dr. Jens Rolff in the institute of Biology at Freie Universität Berlin.

1st Reviewer: Prof. Dr. Jens Rolff

2nd Reviewer: Prof. Dr. Hans-Joachim Pflüger

Date of defense: 10.10.2018

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General introduction

Ecologically based divergent natural selection is a major driver of biodiversity (West-Eberhard, 1989; Stroud & Losos, 2016). Evolutionary biologists believe that ecological differences triggers the early stage of speciation. One of the important ecological factor is the predation, which has strong effects on prey population dynamics and community structure (Sih, 1987; Lima & Dill, 1990) and drives prey trait diversifications (Vamosi, 2005; Schluter, 2009). These effects on prey come not only from the direct killing of predator, but also from the non-consumptive effects of predator (Bolnick & Preisser, 2005; Davenport et al., 2014). In recent years, non-consumptive effects of predators, such as chronic stress, are being appreciated (Clinchy et al., 2013). Non-consumptive pressure of predator significantly influences prey traits and life-histories (Lima & Dill, 1990; Lass & Spaak, 2003; Benard, 2004; Mitchell et al., 2017).

Predator driven trait diversification is widely studied in theoretical and empirical work (reviewed in Vamosi, 2005). Theoretical models showed that sympatric prey may experience trait diversification from interactions with predators (Holt, 1977; Brown & Vincent, 1992; Abrams, 2000). For example, if the antipredator traits of prey are only effective against one predator species but not another predator, divergence will be the most likely outcome (Abrams, 2000). For empirical work, the prominent examples are the guppy (Reznick & Endler, 1982; Rodd & Reznick, 1997; Reznick et al., 2008; Torres-Dowdall et al., 2012), stickleback (Vamosi & Schluter, 2002; Reimchen & Nosil, 2004; Vamosi & Schluter, 2004; Kim & Velando, 2015), and *Enallagma* (McPeek, 1990a; McPeek et al., 2001; Stoks et al., 2003; Stoks & McPeek, 2003) systems. Other organisms such as amphibians (McCollum & Leimberger, 1997; Richardson, 2002; Relyea, 2004; Chandler et al., 2016), daphnia (Tollrian, 1995; Ma et al., 2016), mayflies (Dahl & Peckarsky, 2002), lizards (Brock et al., 2015) and snails (Hoverman et al., 2005) also showed trait diversification in response to predators. Among those examples, changes of predator pressures are caused either by the presence or absence of predators, or by the existence of different predator assemblages. In organisms mentioned above, predation induced various character shifts in defensive traits and consequently, isolation of populations or species according to the presence of predators. Isolation of populations or species become fixed, when transplant of prey populations from low to high predation locations failed because of high mortality (Reznick et al., 2008). Prey populations may undergo rapid evolution of various traits, which can happen, for example, in several months (habitat selection behavior in lizards, Losos et al., 2004), 2.5 years (male coloration in guppy, Reznick et al., 2008) or 26-36 generations (escape behavior in guppy, O'Steen et al., 2002). Trait diversification may first be achieved by phenotypic plasticity and subsequently canalized by genetic changes (West-Eberhard, 1989). Thus, understanding predation-prey interaction is vital to comprehend the diversity of natural organisms.

Antipredator traits and their correlation

Antipredator traits are widespread forms of phenotypic diversification and include defenses in behavior, morphology, and physiology (Benard, 2004). These antipredator traits can fall into two types: (1) pre-contact antipredator traits: avoidance of predatory encounters and (2) post-contact antipredator traits: escaping or fighting after encountering predators (Langerhans, 2007). Pre-contact antipredator traits reduce the probability of detection by a predator, whereas post-contact antipredator traits reduce the chance of consumption after detection by a predator (Fig. 1).

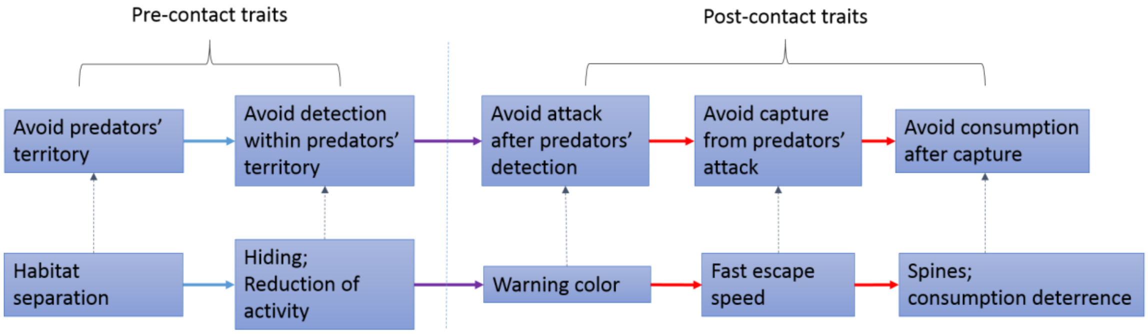

Fig. 1 Pre-contact and post-contact antipredator traits from prey. The second line provides examples for different types of antipredator traits (Langerhans, 2007).

Main antipredator traits and their functions

Antipredator behavior is an extremely labile trait (Scheiner, 1993). In general, under the threaten of predation, prey reduce activity and foraging, increase vigilance or take more use of refuge (Lima & Dill, 1990; Dewitt et al., 1999; Brock et al., 2015). Animals can adjust their behavior constantly according to their environment, which can also make behavior an effective antipredator trait against novel predators (e.g. invasive predators) (Mennen & Laskowski, 2018; Wilson et al., 2018). However, antipredator behavior patterns can also evolve after long-time adaptation to predators (Losos et al., 2004; Stoks & McPeek, 2006). In the absence of certain predators in habitat, prey may lose the ability to recognize those predators (Stoks et al., 2003; Blumstein & Daniel, 2005). Thus, antipredator behaviors play essential roles in prey evolution and adaptations (Abrams & Matsuda, 1997).

Morphological defenses typically consist of adaptations involving shape, structure, color, pattern and size. Warning colors or camouflage can prevent prey from a predator attack (Stevens & Ruxton, 2012; Tan et al., 2017). Morphological structures, such as spines in insects (Johansson & Mikolajewski, 2008), thicker shells in gastropods (Bourdeau et al., 2015), or alteration of body shape in fish (Price et al., 2015) and amphibian larvae (Relyea, 2001a), can increase the handling time for predator, and consequently reduce the mortality of prey after predators' attack.

Predator-induced physiological responses have strong effects on prey life history (Beckerman et al., 2007; Denver, 2009) and growth rate (Stoks et al., 2012; Allen et al., 2016). These influences may come from altered nutrient dynamics in the body, metabolic rates or efficiency of energy utility under predator stress (Stoks et al., 2005; Hawlena & Schmitz, 2010a; Van Dievel et al., 2015). Physiological stress from predators may induce physiological damage on prey (e.g. oxidative damage in Janssens & Stoks, 2013) and thus may lead to risk-induced mortality on prey species (Zanette et al., 2011; MacLeod et al., 2018).

Correlation between anti-predator traits

Preys always apply multiple traits to survive from predators (Benard, 2004; Schmitz, 2017). Antipredator traits, such as activity, morphology and physiological responses are usually highly integrated (Bourdeau & Johansson, 2012; Schmitz, 2017). Activity such as foraging behavior and habitat choice have a strong influence on the availability of food resources, and, thus are closely correlated with growth rate (Werner & Anholt, 1993). Behavior like swimming ability is dependent on both morphology (McCollum & Leimberger, 1997) and physiological traits (Mikolajewski et al., 2010). Dewitt et al. (1999) defined four functional relationships between behavior and morphology, which can be extended to relationships among all antipredator traits: (1) codependence, which means that traits are structurally linked; (2) complementation, which indicate traits are structurally independent but must be used in combinations to be effective against predators; while for non-functionally related antipredator traits, the relationship can be (3) specialization (statistically positive correlation) or (4) compensation (statistically negative correlation). Thus, in order to understand natural selection on antipredator traits, it is critical to consider correlations among antipredator traits (Hoverman et al., 2005).

Fitness of trade-offs in antipredator traits

Antipredator traits directly relate to the fitness of prey species (Lind, 2005; Baxter-Gilbert et al., 2018). Successful antipredator traits aid prey to avoid predators or escape from capture, which will reduce the mortality of prey. However, antipredator traits also impose pervasive fitness costs (Skelly, 1992; Tollrian, 1995; Peckarsky & McIntosh, 1998; Lagergren et al., 2001). The most familiar case concerns a fitness trade-off between foraging behavior and encounter rate with predators (Anholt & Werner, 1995). Moreover, fitness trade-offs can also arise from energy allocation (Brodie, 1999; Mangel & Stamps, 2001) or from the situation that one defensive trait is effective against one predator but does not work against the other possible enemies (Mikolajewski et al., 2006; Edeline et al., 2008).

Inter- and intra-specific evolution in antipredator traits

Antipredator traits can differ dramatically among prey species and conspecific geographic populations (Peckarsky et al., 2001; Relyea, 2001a). Examining divergence among prey populations or species enable researchers to discriminate the ecological forces creating the diversity and further understand how ecological forces drive divergence (Schluter & McPhail, 1992; Robinson et al., 1996; DeWitt et al., 2000). Moreover, intraspecific variation can be as strong as interspecific variation (Hélène et al., 2010; Bestion et al., 2015). Reduction in foraging behavior in response to predators can be found both among populations (Stoks et al., 2012) and among species (Stoks et al., 2003) in damselflies. Burst escape speed in *Leucorrhinia* species follows a similar pattern among populations and among species (Mikolajewski et al., 2010).

For trait divergence across species, phylogenetic constrains should be considered because species do not represent independent samples (Felsenstein, 1985). The influence of shared ancestry on trait divergence can be assessed by comparative studies (Pagel & Harvey, 1989; Freckleton et al., 2002). In performing comparative studies, three criteria must be satisfied: 1) data should be collected from a system of species, 2) the phylogenetic relationship among species should be known, 3) the species used should experience different selection regimes. Usually phylogenetic signal (either Pagel's λ or K_{mult}) must also be detected from the data set. Although there are debates on comparative methods (Harvey et al., 1995; Westoby et al., 1995), phylogenetic dependence does exist within a wide range of phylogenies and data (Freckleton et al., 2002).

Phenotypic variation among populations or species can stem from either genetic changes or phenotypic plasticity (Rundle & Nosil, 2005; Pfennig et al., 2010). It is no doubt that a genetic mutation can change certain genotype and may thus induce phenotype change (Voskarides, 2017). While without genetic change, individuals can still express different phenotypes because of adaptation to distinctive environments, which is defined as phenotypic plasticity (West-Eberhard, 1989; Pigliucci, 2001; Pfennig et al., 2010). Furthermore, phenotypic divergence from both genetic change and phenotypic plasticity, will promote lineage differentiation and ultimately speciation (Schluter, 2009; Hoso et al., 2010; Pfennig et al., 2010; Higham et al., 2016).

Freshwater habitats and study organisms

Freshwater environments include habitats with different top predators (Wellborn et al., 1996). Permanent lakes are dominated by predatory fish, such as perch, pike, crucian carp or common roach; while some lakes do not have fish but instead are dominated with large invertebrate predators, such as large dragonfly larvae or waterbugs. Many predatory fish species search for their prey while moving (Ehlinger, 1989; McPeek, 1990a), whereas invertebrate predators usually follow a sit-and-wait strategy to ambush prey (Wellborn et al., 1996). The existence of these different predator assemblages strongly shapes the distribution of freshwater fauna (Wellborn et al., 1996; Petrin et al., 2010; Dijkstra et al., 2014). Moreover, comparative isolation of freshwater areas also makes freshwater habitats as another natural laboratory for studying species diversification like islands (Whittaker & Fernández-Palacios, 2007; Emerson, 2008; Runemark et al., 2014).

Fig. 2 Freshwater habitat of *Leucorrhina* species (Photo taken on 24th, Jun. 2016 in Barschsee)

In this thesis, I studied all five European *Leucorrhinia* (Odonata: Libellulidae) species, which is an ideal model for studying the evolutionary ecology of antipredator trait diversification among species (Johansson & Mikolajewski, 2008). Larval *Leucorrhinia* occupy the important intermediate positions in the food web of freshwater habitats (Johnson, 1991). Distributions of *Leucorrhinia* larvae are strongly shaped by predator type (Petrin et al., 2010): *L. albifrons, L. caudalis,* and *L. pectoralis* usually inhabit lakes with predatory fish as top predator (hereafter fish lakes for this kind of lake; fish-lake species for this group of species), whereas *L. dubia* and *L. rubicunda* prefer lakes without fish but with large predatory dragonfly predators (hereafter dragonfly lakes for this kind of lake; dragonfly-lake species for this group of species). Further, the phylogenetic relationship of *Leucorrhinia* species is well-resolved, which suggests that fish-lake species representing the ancestral state (Hovmöller & Johansson, 2004). In contrast to fish-lake species, dragonfly-lake species adopt suites of antipredator traits because of top-predator change, such as reduced spines length (Mikolajewski & Johansson, 2004), reduced burst escape speed, decreased arginine kinase activity (Mikolajewski et al., 2010) and decreased phenotypic integration of abdominal traits (Mikolajewski et al., 2015b).

As adult dragonflies are capable of dispersing over long distance, habitat shifts are common among different generations of dragonfly larvae (Pajunen, 1962; McCauley, 2006). Thus, *Leucorrhinia* species such as *L. pectoralis* expand from fish lakes into dragonfly lakes where they might experience different selection pressures associated with changes in predation regimes (Blumstein et al., 2004; Runemark et al., 2014). This can lead to a dramatic change in expression of antipredator traits (McPeek, 1990a; Relyea, 2003; Stoks & McPeek, 2006), because larvae which hatch in a non-preferred habitat will be wiped out if they do not possess any antipredator traits against novel predators (Gurevitch & Padilla, 2004; Losos et al., 2004). Therefore, predation regime shift of certain *Leucorrhinia* larvae provides another opportunity to study the early stage of lineage diversification.

Fig. 3 A *Leucorrhinia* larvae living on aquatic plants (Photo taken on 17th, Aug. 2017 in my larvae raising bucket)

Objective and outline

The main aim of this thesis is to investigate how prey responds to predation regime shifts. On one hand, we focused on different antipredator traits adopted by prey. Although several species have been intensively studied (as mentioned above), unique life history and characters will show a different view of antipredator traits. Thus, from this part we want to show how prey integrated all antipredator traits together and improved their fitness to different predators (**Chapter I, II, III**). On the other hand, I want to disentangle predator effects in different levels, i.e., species level (**Chapter I, II, III**) and population level (**Chapter IV**). Studies on these two levels will improve the understanding of predators' roles on driving prey divergence.

From **Chapter I to chapter III**, I focused on traits divergence among species from fish lakes and dragonfly lakes. I compared a series of antipredator traits in these two *Leucorrhinia* species groups. In **chapter I**, I investigated the antipredator behaviors, i.e., swimming, walking, foraging behaviors and food capture behaviors, in penultimate- and final-instar larvae. From this, I want to clarify how the behavior evolved after *Leucorrhinia* species shift from fish lakes to dragonfly lakes. Moreover, I also examined the plastic responses of different species groups. The mechanism of behavior trade-off was further discussed. In **chapter II**, I studied the evolution of intercorrelated traits to burst escape swimming. As fish-lake species have faster burst escape speed (Mikolajewski et al., 2010) than dragonfly-lake species, traits related with burst escape behavior can also differ. Here we measured body shape, branchial chamber size and muscle mass of the abdomen in both *Leucorrhinia* species groups. The results showed that predation regimes shift changed burst escape swimming and its intercorrelated traits as well. In **Chapter III**, I reported the growth rate and digestive traits in both *Leucorrhinia* species groups. Growth rate can be changed by predator shift via behavioral changes or physiological changes. As behavioral changes will be discussed in chapter I, I further assessed how growth rate changes among two *Leucorrhinia* groups related to physiological changes in digestive traits.

In **Chapter IV**, I changed the focus from species divergence to population diversification. Final instar exuviates of *L. pectoralis* were used in this part. I measured dorsal and lateral spine length and body size of exuviates from both fish lakes and dragonfly lakes. I disentangled whether predation shift changes both mean and variance of prey traits among populations. Skewness of trait distribution were also calculated and discussed.

In **Chapter V**, I applied developmental approaches in order to gain a more complete understanding of predator-induced antipredator spines. In this part, larvae were collected at continuous life-stages and spine length was measured. By applying non-linear growth models on the growth of spines, I want to clarify whether *Leucorrhinia* species from different predator regime habitats evolved different developmental constraints on elongation of antipredator spines.

Chapter I

Shift in predation regime mediates diversification of foraging behavior in a dragonfly genus[1]

https://doi.org/10.1111/een.12530

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[1] This work was published in *Ecological Entomology* in 2018.

Chapter II

Changing the habitat: the evolution of intercorrelated traits to escape from predators[2]

https://doi.org/10.1111/jeb.12879

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[2] This work was published in *Journal of Evolutionary Biology* in 2016. The contributions of Bin Jiang include measuring muscle mass and branchial chamber size.

2.1 Abstract

Burst escape speed is an effective and widely used behavior for evading predators, with burst escape speed relying on several different morphological features. However, we know little about how behavioral and underlying morphological attributes change in concert as a response to changes in selective predation regime. We studied intercorrelated trait differentiation of body shape and burst-swim-mediating morphology in response to a habitat shift-related reduction in burst escape speed using larvae of the dragonfly genus *Leucorrhinia*. Species in this genus underwent a well-known habitat shift from predatory fish lakes (fish lakes) to predatory fish-free lakes dominated by large predatory dragonflies (dragonfly lakes) accompanied by relaxed selection on escape burst speed. Results revealed that species from fish lakes that possess faster burst speed have evolved a suite of functionally intercorrelated traits, expressing a wider abdomen, a higher abdominal muscles mass and a larger branchial chamber compared with species from dragonfly lakes. In contrast, populations within species did not show significant differences in muscle mass and branchial chamber size between lake types in three of the species. High multi-collinearity among variables suggests that traits have evolved in concert rather than independently when *Leucorrhinia* shifted from fish lakes to dragonfly lakes. Thus, relaxed selection on burst escape speed in dragonfly-lake species resulted in a correlated reduction of abdominal muscles and a smaller branchial chamber, likely to save production and/or maintenance costs. Our results highlight the importance of studying integrated behavioral and morphological traits to fully understand the evolution of complex phenotypes.

Key words: abdominal muscle; branchial chamber; habitat shift; jet propulsion; *Leucorrhinia*; phenotypic integration; phenotypic plasticity; predation; relaxed selection.

2.2 Introduction

Predator occurrence represents an important selective agent driving behavioral and morphological diversity (e.g. Rundle et al., 2003; Langerhans et al., 2004; Stoks & McPeek, 2006; Mikolajewski et al., 2010; Martin et al., 2013). How behavioral and morphological traits respond to changes in selection by predators has been intensively studied (e.g. Relyea, 2001b; Mikolajewski et al., 2006; Marchinko, 2009; Miehls et al., 2014; Wund et al., 2015). Less well understood is how multiple traits that are functionally linked (intercorrelated) respond in concert to changes in the selective regime (but see Marquez & Knowles, 2007). This is important because prey often use multiple rather than single defense mechanisms to avoid and repel predators (e.g. Mikolajewski & Johansson, 2004; Relyea, 2004; Nunes et al., 2014). Ecological communities that display shifts in predation regime and where predator–prey interactions are well studied provide suitable study systems for understanding multiple trait evolution but of few such studies exist (but see McPeek, 1999; Losos et al., 2002; Mikolajewski et al., 2010).

To avoid being killed by predators, prey have evolved multiple consecutive means to avoid encounters with predators (reviewed in Sih, 1987 and Langerhans, 2007). If such avoidance strategies fail and predators attack, most prey rely on post-encounter traits such as rapid escape behavior, which rely on morphological adaptations of prey (reviewed in Sih, 1987 and Langerhans, 2007), for example, long legs and effective fibre tissue to enable fast running (Losos et al., 2002; Scales et al., 2009) or streamlined body shape, larger tails and deeper muscles for fast escape swimming (Dayton et al., 2005; Langerhans, 2009). Thus, selection pressure for rapid escape may cause correlated trait changes among behavioral, physiological, morphological, functional and/or developmental interconnected characters (Travis, 1989; Pigliucci & Preston, 2004; Bourdeau & Johansson, 2012).

As trait changes in morphology that increase escape behavior often affect the entire body shape of an organism, it is interesting to examine how body shape and morphology are associated with predation risk regimes in nature. Body shape has been found to be under strong selection by predators (e.g. McPeek, 1995; Langerhans & DeWitt, 2004; Scharnweber et al., 2013; Nunes et al., 2014; Vega-Trejo et al., 2014). Differentiation of prey body shape in response to predators either stems from direct selection to increase performance (e.g. Langerhans et al., 2004) or represents the indirect by-product of selection on intercorrelated traits (e.g. Johansson & Andersson, 2009). For a thorough understanding of these phenotypic changes in body shape as a response to predation, detailed information about the direction of selection on shape as well as the functional relationships between morphological traits and body shape and how it is related to behaviors such as escape speed is needed.

Assuming a link between micro- and macroevolution, differences in intercorrelated trait expression between species experiencing different predator regimes are expected to be similar comparing populations within a species experiencing the same selective direction and strength. Therefore, population differentiation in, for example, intercorrelated traits can stem from either fixed genetic changes or phenotypic plasticity of genotypes, with both being able to promote lineage differentiation and ultimately speciation (Coyne & Orr, 2004; Pfennig et al., 2010; Nosil, 2012). Indeed, predator-driven differentiation of populations experiencing different predators can result via different mechanisms in speciation (reviewed in Langerhans, 2007). Matching intra- with interspecific pattern of plastic and/or genetically fixed trait expression and trait correlations in response to environmental differences can thereby represent an indication but no proof for intraspecific divergence playing a role in patterns of diversification (Pfennig et al., 2010 and references therein).

Species of the dragonfly genus *Leucorrhinia* (Odonata, Libellulidae) are an excellent model system to study multivariate antipredator trait evolution (reviewed in Johansson & Mikolajewski, 2008). Ancestrally, species occupied lakes dominated by predatory fish (fish lakes) with larvae exhibiting large defensive dorsal and lateral abdominal spines against gape-limited predators (Hovmöller & Johansson, 2004; Mikolajewski & Johansson, 2004; Mikolajewski & Rolff, 2004; Petrin et al., 2010). A habitat-related shift to lakes that lack predatory fish but being dominated by large invertebrate predators (dragonfly lakes) resulted in an evolutionarily reduction in number and length of spines. This was caused by antagonistic selection from large invertebrate predators that selected for loss of defensive spines, because loss of spines results in less effective capture success and lower mortality in these larvae (Hovmöller & Johansson, 2004; Mikolajewski et al., 2006; Petrin et al., 2010).

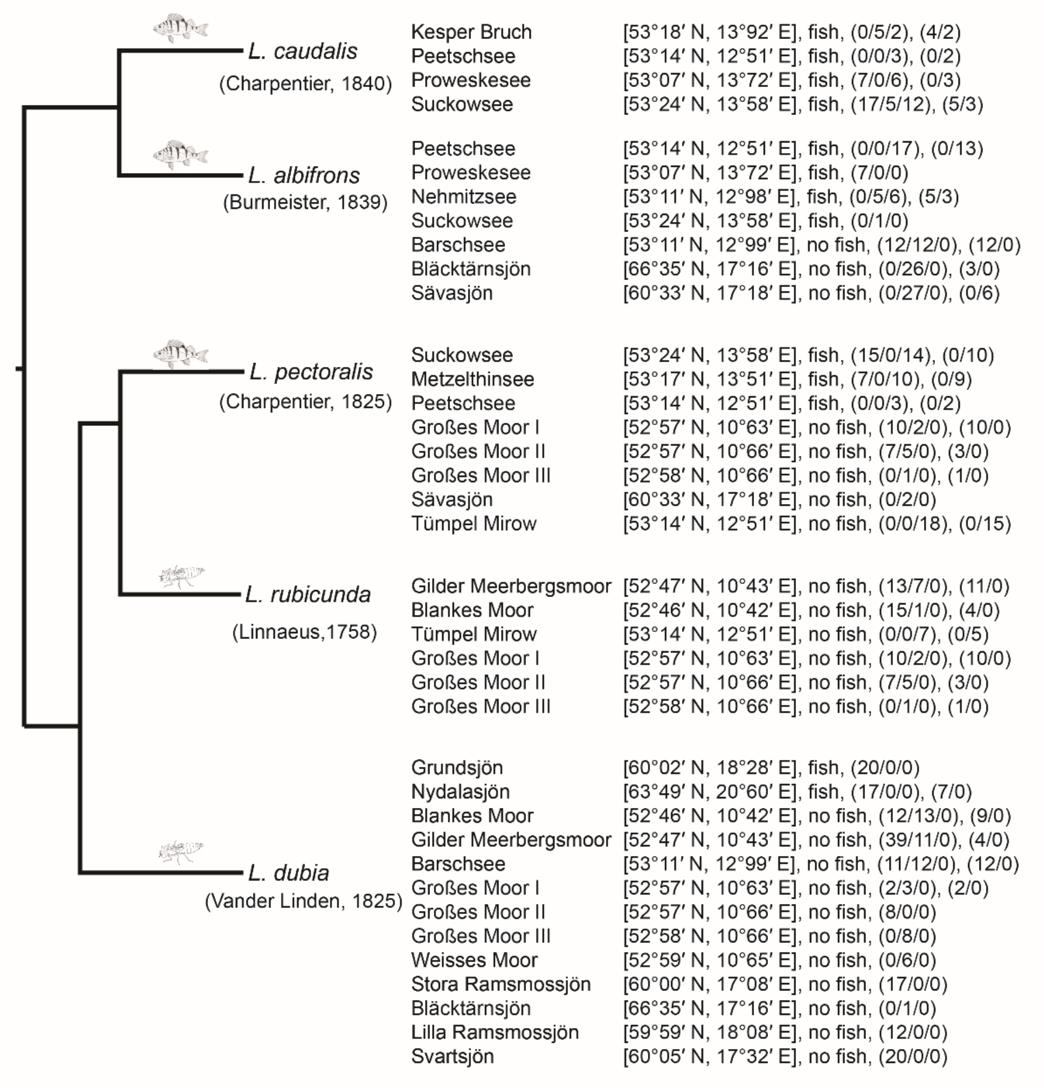
Even though antagonistic selection was found responsible for the loss of spines (Mikolajewski et al., 2006), overall species in dragonfly lakes experience relaxed selection (Mikolajewski et al., 2010, 2015a). This is due to invertebrate predators following a sit-and-wait strategy and do not pursue prey in comparison with predatory fish resulting a lower capture success and perceived predation risk (Stoks & De Block, 2000; Mikolajewski & Rolff, 2004; Mikolajewski et al., 2006). Relaxed selection in dragonfly lakes resulted in a reduction in burst escape speed in dragonfly-lake species (Mikolajewski et al., 2010). However, how changes in the selective regime accompanied by relaxed selection affect the underlying intercorrelated morphological traits mediating burst escape speed is not studied yet. Unique in insects, all anisopteran larvae use burst escape swimming to escape predators via jet propulsion (Corbet, 1999). Larvae contract water through the anus from a special rectal branchial chamber via contraction of large dorso-ventral and longitudinal abdominal muscles (Mill & Pickard, 1975). Muscle contraction results in a short and strong expulsion of water, propelling the animal in the opposite direction (Trueman, 1980). Muscle tissue is highly costly to produce and maintain (Zera & Denno, 1997; Marden, 2000). Because relaxed selection on escape swimming resulted in a reduced burst speed in dragonfly-lake species (Mikolajewski et al., 2010), we expect (a) species from dragonfly lakes to save costs by reducing muscle mass. This is supported by previous findings that arginine kinase activity is reduced in species invading dragonfly lakes, an enzyme which provides insect muscle tissue rapidly with ATP for short lasting, energetically costly exercise (Mikolajewski et al., 2010 and references within). In addition, we expect (b) a reduction in muscle mass in dragonfly-lake species to indirectly affect the larval body shape by dragonfly species possessing a narrower abdomen than fish-lake species, as a response to the lower space required by reduced muscle tissue. In some *Leucorrhinia* species, there are indications for morphological and/or behavioral population differentiation in the same direction as found interspecifically with regard to differences in the predation regime (Arnqvist & Johansson, 1998; Mikolajewski & Johansson, 2004; Mikolajewski et al., 2010, 2015a). We therefore expect that (c) the interspecific differences in intercorrelated traits are mirrored among populations within species.

To understand how changes in predation regime have affected intercorrelated traits in the *Leucorrhinia* dragonfly larvae, we studied the following variables: body shape, branchial chamber size and abdomen muscle mass as well as how all three morphological traits correlate with burst escape speed. We hypothesize that (1) the documented reduction in burst escape swimming when *Leucorrhinia* species invaded dragonfly lakes (Mikolajewski et al., 2010) resulted in a correlated narrower abdomen in comparison with fish-lake species; (2) the narrower abdomen in dragonfly-lake species stems from a reduction in muscle mass rather than smaller branchial chamber in order to save energy costs; (3) the change in body shape and muscle mass correlates with the documented reduction in burst escape speed; and (4) the interspecific differences in body shape and muscle mass should be mirrored in population differentiation in three of our study species that occasionally occur in opposing predator regime.

2.3 Materials and methods

2.3.1 Study system and sampling

The genus *Leucorrhinia* comprises ~14 species worldwide (Hovmöller & Johansson, 2004). Here, we concentrate on the five European species: *L. albifrons*, *L. caudalis*, *L. dubia*, *L. pectoralis* and *L. rubicunda* (Fig. 1). All five species overlap in their distribution in Central Europe (Askew, 2004). Larvae hatch shortly after oviposition in late spring and early summer, undergoing a 1- to 3-year larval period depending on latitude. *Leucorrhinia albifrons*, *L. caudalis* and *L. pectoralis* are classified as fish-lake species, and they are heavily armoured by lateral and dorsal abdominal spines (Johansson & Mikolajewski, 2008; Petrin et al., 2010). Dragonfly-lake specialists *L. dubia* and *L. rubicunda,* on the other hand, have shorter lateral and anterior dorsal abdominal spines, and they lack posterior dorsal spines (Johansson & Mikolajewski, 2008; Petrin et al., 2010). Selection against spines by invertebrate predators that grasp prey is stronger on posterior spines than on anterior spines, because the dorsal anterior spines are covered by the enlarged wing pads in later instar larvae (Petrin et al., 2010). Note that three of the *Leucorrhinia* species, the fish-lake specialist *L. albifrons* and *L. pectoralis* as well as the dragonfly-lake specialist *L. dubia* occasionally also occur in the opposing habitat, albeit in low numbers (Johansson & Brodin, 2003; Mikolajewski & Johansson, 2004; Petrin et al., 2010; Mikolajewski & Johansson, pers. observation).



L. caudalis (Charpentier, 1840)	Kesper Bruch	[53°18′ N, 13°92′ E], fish, (0/5/2), (4/2)
	Peetschsee	[53°14′ N, 12°51′ E], fish, (0/0/3), (0/2)
	Proweskesee	[53°07′ N, 13°72′ E], fish, (7/0/6), (0/3)
	Suckowsee	[53°24′ N, 13°58′ E], fish, (17/5/12), (5/3)
L. albifrons (Burmeister, 1839)	Peetschsee	[53°14′ N, 12°51′ E], fish, (0/0/17), (0/13)
	Proweskesee	[53°07′ N, 13°72′ E], fish, (7/0/0)
	Nehmitzsee	[53°11′ N, 12°98′ E], fish, (0/5/6), (5/3)
	Suckowsee	[53°24′ N, 13°58′ E], fish, (0/1/0)
	Barschsee	[53°11′ N, 12°99′ E], no fish, (12/12/0), (12/0)
	Bläcktärnsjön	[66°35′ N, 17°16′ E], no fish, (0/26/0), (3/0)
	Sävasjön	[60°33′ N, 17°18′ E], no fish, (0/27/0), (0/6)
L. pectoralis (Charpentier, 1825)	Suckowsee	[53°24′ N, 13°58′ E], fish, (15/0/14), (0/10)
	Metzelthinsee	[53°17′ N, 13°51′ E], fish, (7/0/10), (0/9)
	Peetschsee	[53°14′ N, 12°51′ E], fish, (0/0/3), (0/2)
	Großes Moor I	[52°57′ N, 10°63′ E], no fish, (10/2/0), (10/0)
	Großes Moor II	[52°57′ N, 10°66′ E], no fish, (7/5/0), (3/0)
	Großes Moor III	[52°58′ N, 10°66′ E], no fish, (0/1/0), (1/0)
	Sävasjön	[60°33′ N, 17°18′ E], no fish, (0/2/0)
	Tümpel Mirow	[53°14′ N, 12°51′ E], no fish, (0/0/18), (0/15)
L. rubicunda (Linnaeus,1758)	Gilder Meerbergsmoor	[52°47′ N, 10°43′ E], no fish, (13/7/0), (11/0)
	Blankes Moor	[52°46′ N, 10°42′ E], no fish, (15/1/0), (4/0)
	Tümpel Mirow	[53°14′ N, 12°51′ E], no fish, (0/0/7), (0/5)
	Großes Moor I	[52°57′ N, 10°63′ E], no fish, (10/2/0), (10/0)
	Großes Moor II	[52°57′ N, 10°66′ E], no fish, (7/5/0), (3/0)
	Großes Moor III	[52°58′ N, 10°66′ E], no fish, (0/1/0), (1/0)
L. dubia (Vander Linden, 1825)	Grundsjön	[60°02′ N, 18°28′ E], fish, (20/0/0)
	Nydalasjön	[63°49′ N, 20°60′ E], fish, (17/0/0), (7/0)
	Blankes Moor	[52°46′ N, 10°42′ E], no fish, (12/13/0), (9/0)
	Gilder Meerbergsmoor	[52°47′ N, 10°43′ E], no fish, (39/11/0), (4/0)
	Barschsee	[53°11′ N, 12°99′ E], no fish, (11/12/0), (12/0)
	Großes Moor I	[52°57′ N, 10°63′ E], no fish, (2/3/0), (2/0)
	Großes Moor II	[52°57′ N, 10°66′ E], no fish, (8/0/0)
	Großes Moor III	[52°58′ N, 10°66′ E], no fish, (0/8/0)
	Weisses Moor	[52°59′ N, 10°65′ E], no fish, (0/6/0)
	Stora Ramsmossjön	[60°00′ N, 17°08′ E], no fish, (17/0/0)
	Bläcktärnsjön	[66°35′ N, 17°16′ E], no fish, (0/1/0)
	Lilla Ramsmossjön	[59°59′ N, 18°08′ E], no fish, (12/0/0)
	Svartsjön	[60°05′ N, 17°32′ E], no fish, (20/0/0)

Fig. 1 Modified phylogeny of the 5 European *Leucorrhinia* species from Hovmöller & Johansson (2004). Picture of fish and dragonfly larvae indicate fish-lake or dragonfly-lake specialist. For each species, sample location with coordinates is given and indicated whether the lake contained fish or was fish-free. The first brackets denote sample size from consecutive years 2012/2013/2014 for the body shape analysis. The second brackets give sample size for measurements on branchial chamber size and muscle mass from a subset of larvae in 2013/2014 (see methods).

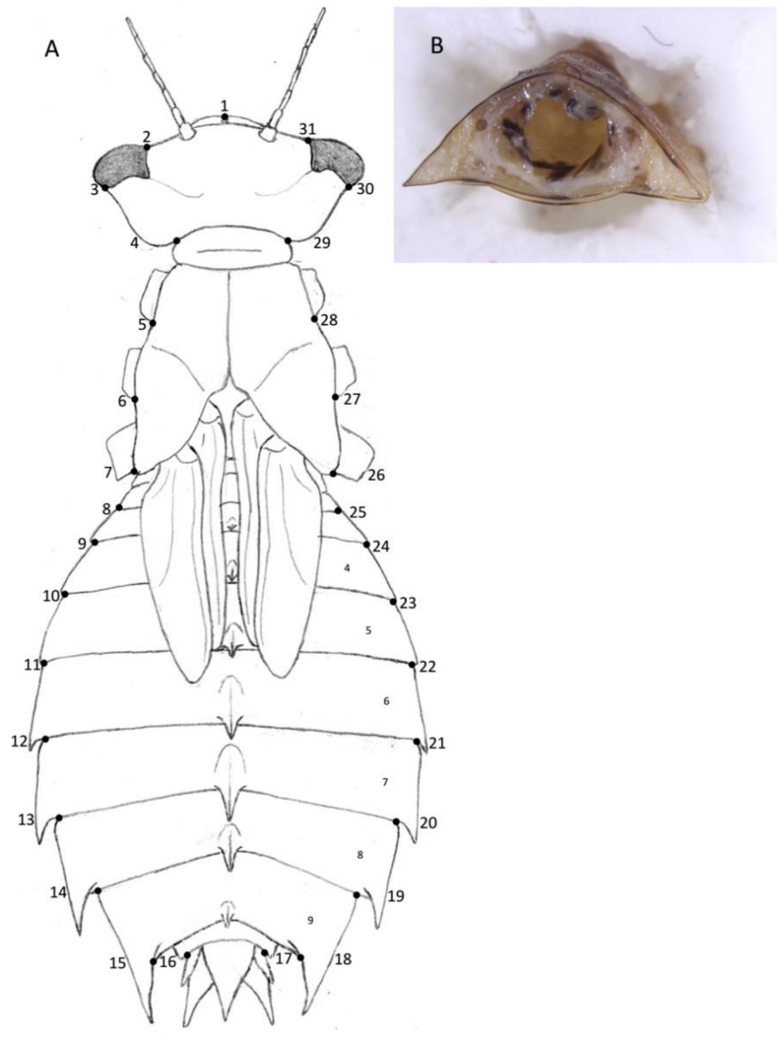


Fig. 2 (a) Dorsal view of a larval *Leucorrhinia caudalis* with indicated position of the 31 digitized landmarks used in the morphometric shape analysis. Numbers on abdominal segments denote counting of segments in odonate larvae. Drawing by Axel Conrad. (b) Cross section of a larval *Leucorrhinia dubia* at abdominal segment 7. Picture shows the wide-open branchial chamber with the black gills inserting into the lumen.

Last instar larvae of each species were sampled from a minimum of four separate populations in consecutive early springs of 2012-2014 in Northern Germany and Sweden (sample size and location see Fig. 1) and preserved individually in 80% ethanol. Fish lakes all contained at least perch (*Perca fluviatilis* L.), whereas dragonfly lakes all were occupied by large aeshnid larvae of the genera *Aeshna* and/or *Anax* (Odonata: Anisoptera). Both predator types are known to cause substantial selection pressure on behavioral, morphological, physiological and life-history traits in odonate larvae including our study species (reviewed in Johansson & Stoks, 2005; Stoks & McPeek, 2006; Johansson & Mikolajewski, 2008). Larvae were brought to the laboratory and digital photographs of each larva were taken from the dorsal and lateral side using a dissection scope. From photographs, we determined overall body length, which was measured from the tip of the labium to the end of abdominal segment 10 (Mikolajewski et al., 2015b), using the open-source program ImageJ 1.46o (National Institutes of Health, Bethesda, MD, USA). Numbers of larvae used in the analyses are given in Fig. 1.

2.3.2 Body shape

To investigate the body morphology of individual larvae, we used a landmark-based geometric morphometric approach (Bookstein, 1991). Digital dorsal photographs were transferred to TPSdig2 (all TPS programs http://life.bio.sunysb.edu/morph), and we placed 31 homologous landmarks on the body (Fig. 2a). All landmarks were set from the same person to minimize measurement bias. Furthermore, we checked for outliers using the 'Find outliers' function of the open source software MorphoJ (Klingenberg, 2011), in all analyses described below. As dragonfly larvae are bilaterally symmetric, we paired 15 of those landmarks with their symmetric opponent (except for landmark 1, the anterior tip of the head) and used the computational procedure implemented in MorphoJ to analyse object symmetry (Klingenberg et al., 2002). In short, this procedure corrects for the partially redundant information of symmetric structures that potentially lead to statistical problems by partitioning the shape variation into symmetric and asymmetric components of the original configurations and their images mirrored along the midline (Mardia et al., 2000; Klingenberg et al., 2002). For our purposes, we primarily focused on the symmetric component of shape variation. We removed nonshape variation, such as variation in location and orientation using Procrustes Superimposition (Rohlf & Slice, 1990). For subsequent analyses, we extracted the scores of the first and second PCA axes (PC1 and PC2) which explained ~69% of the variation. To visualize shape differences between dragonfly- and fish-lake species, we used discriminant function analyses (DFA) implemented in MorphoJ (see Fig. 3c).

2.3.3 Branchial chamber and muscle mass

To investigate the branchial chamber size and abdomen muscle mass, we measured chamber cross-sectional length and extracted muscle mass from the abdomen, respectively. The branchial chamber for water ejection in anisopteran larvae is located between abdominal segments 6 and 10 (Hughes & Mill, 1966). As we were unable to measure total volume of the branchial chamber, we measured area of cross sections at the widest point of the branchial area at the anterior end of segment 7 (pers. observation, Fig. 2b) as a proxy for size. To do this, we prepared cross sections for a subset of larvae (see Fig. 1) by cutting the abdomen between segments 6 and 7 with a scalpel and took digital photographs. Area of the branchial chamber from photographs was measured using ImageJ 1.46o.

Abdominal contraction by muscles during burst escape swimming involves movements of abdominal segments 2–10 (Pickard & Mill, 1974). However, main muscle activity for burst escape swimming is located between segments 5 and 10 (Mill & Pickard, 1975) (Fig. 2). Thus, we extracted muscle mass from segments 5–10 of the same larvae as we measured branchial chamber size. We extracted muscle mass by first extracting fat body from the abdominal sections, rinsing individuals for 48 h separately in 2-mL microtubes containing 1.5 mL dichlormethane (Mikolajewski et al., 2013, 2015b). Microtubes were gently moved by a laboratory shaker during extraction and dried afterwards for 48 h at 60 ℃ and weighed to the nearest 0.01 mg. We continued with extracting muscle mass by following the protocol of Marden (1987). In short, abdominal sections were placed individually in microtubes filled up with 1.5 mL NaOH (0.35 mol/L) for 24 h, again gently moved by a laboratory shaker. After this procedure, we rinsed sections with distilled water and dried them for 48 h at 60 ℃, again weighing them to the nearest 0.01 mg. Muscle content was estimated by subtracting the dry mass after extraction of muscles from the dry mass before extraction of muscles (Mikolajewski et al., 2004).

2.3.4 Burst speed

To explore whether trait differentiation in body shape, branchial chamber size and muscle mass is evolutionarily linked to habitat shift-related reduction in burst escape speed (see above), we extracted data on burst escape speed for the 5 *Leucorrhinia* species from a previous publication (Mikolajewski et al., 2010). In short, bust speed was estimated from a simulated predator attack that elicited a burst swim escape response in the *Leucorrhinia* larvae.

2.3.5 Statistical analyses

All analyses were performed using R 3.2.1 (R Core Team 2015). To take into account sampling set-up, we fitted linear mixed-effects models (package lme4, Bates et al., 2015) to control for different sampling years and the hierarchical structure of predator and species occurrence across different habitats (Mikolajewski et al., 2015b). We fitted a multivariate model for the extracted PC1 and PC2 scores of body shape and separated univariate models for branchial chamber size and muscle mass using restricted maximum-likelihood estimates with species as a fixed effect, sample year as a random effect and sample location nested within species as an additional random effect (Mikolajewski et al., 2010, 2015a). We corrected measures on all traits for body size differences among species using body length as an additional additive covariate. Data on branchial chamber size were log transformed and data on muscle mass cube-root-transformed to achieve normality of residual error structure. Because the larval branchial chamber is elastic, branchial chambers in the dead larvae differed in their level of contraction between wide-open, almost closed and an intermediate state. To control for this additional variance, we added contraction level (wide open/half open/closed) as a random effect analysing branchial chamber size. From each model, we extracted least-squares means (package lsmeans, Lenth, 2015) for all species and used them in subsequent inter- and intraspecific analyses (Mikolajewski et al., 2010, 2015a, b).

a) Interspecific analyses

To control for phylogenetic dependency and to determine whether body shape, branchial chamber size and muscle mass evolved in a correlated response with burst escape speed to a habitat shift-related change in predation regime, we applied phylogenetic principal component analysis on the correlation matrix of the four variables (package phytools 0.5–00, Revell, 2012). Only the first extracted phylogenetic principal component had an eigenvalue > 1 (see Results), and scores of this phylogenetic principal component were used as dependent variable in a subsequent phylogenetic ANOVA (Garland et al., 2005) with predator regime (fish-lake vs. dragonfly-lake) as independent variable (Revell, 2012). We used 10 000 simulations to obtain P-values.

For all phylogenetic analyses, branch length was incorporated from the current *Leucorrhinia* phylogeny (Hovmöller & Johansson, 2004; Hovmöller pers. communication, Fig. 1).

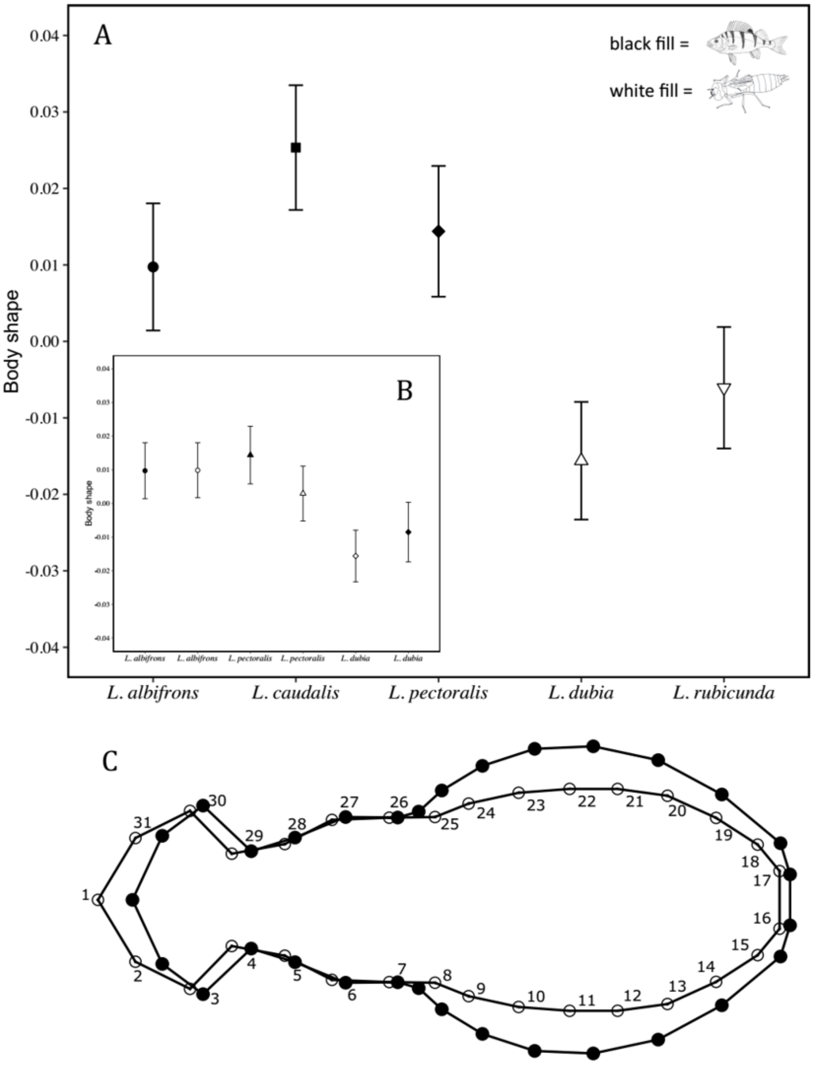


Fig. 3 LS means for body shape from a multivariate model of combined principal components (PC) 1 of the same geometric morphometric shape analysis (see methods). (a) Presented are means (±SE) of all 5 *Leucorrhinia* species. Black filling denotes fish-lake specialists and white filling dragonfly-lake specialists. (b) Fish-lake specialist *Leucorrhinia albifrons* and *L. pectoralis* as well as dragonfly-lake specialist *L. dubia* can also occur in low numbers in the opposite habitat (e.g. *L.*

dubia in fish lakes). Presented are means (±SE) of these three species with black filling denoting individuals from fish lakes and white denoting individuals filling from dragonfly lakes. (c) Warped outline drawings from a discriminant function analyses depicting the average shape of individuals from fish lakes (black filling), compared to the average shape of individuals from dragonfly lakes (white filling). Shape changes were amplified 5 times to facilitate visualization.

b) Intraspecific analyses

To test for intraspecific differences in body shape, branchial chamber size and muscle mass among larval *L. albifrons*, *L. dubia* and *L. pectoralis* from fish and dragonfly lakes, we compared individuals of the three species from their preferred habitat (e.g. *L. dubia* from dragonfly lakes) with individuals sampled from the non-preferred habitats (e.g. *L. dubia* from fish lakes). Within these models, we tested for specific a priori predictions using a set of three linear contrasts (package lsmeans, Lenth, 2015). We tested for intraspecific differences in body shape, branchial chamber size and muscle mass within the fish-lake specialists (i) *L. albifrons* and (ii) *L. pectoralis* as well as the dragonfly-lake specialist (iii) *L. dubia*. Multiple *P*-values were adjusted using Sidak method. As intraspecific data on burst speed for the three *Leucorrhinia* species are not available, we did not correlate morphological data with burst speed.

2.4 Results

2.4.1 Body shape

Principal component 1 explained 52.37% and principal component 2 explained 16.86% of the total variances. Figure 3c shows the average shape of individuals from fish lakes compared to the average shape of individuals from dragonfly lakes. These differences are primarily associated with a wider abdomen of segments 3–8 (landmarks 8–15 and 18–25) and a shorter head (landmarks 1–3 and 1–30) in individuals from fish lakes (Fig. 3c).

2.4.2 Interspecific differences

Of the four components extracted by the phylogenetic principal component analysis, phylogenetic principal component 1 (phyl-PC1) almost captured all of the variance (97%, eigenvalue: 3.886). Traits loaded high and positive on phyl-PC1 (body shape = 0.983, branchial chamber size = 0.979, muscle mass = 0.993, burst speed = 0.988), indicating high multi-collinearity among variables with traits changing in concert rather than independently.

Species from fish lakes (*L. albifrons* = 0.186, *L. caudalis* = 0.415, *L. pectoralis* 0.476) had significantly higher phylogenetic PC1 scores than species from dragonfly lakes (*L. dubia* = 0.476, *L. rubicunda* = 0.345) (phylogenetic ANOVA (PC1): $F_{1,3}$ = 51.78, *P* = 0.001). This means that species from fish lakes have a faster burst speed correlated with a wider abdomen and shorter head, and a larger branchial chamber as well as greater muscle mass than species from dragonfly lakes (Figs 3a and 4a).

2.4.3 Intraspecific differences

Body shape did not differ in fish-lake specialists *L. albifrons* (t = 0.03, d.f. = 27.31, *P* = 1.000) and *L. pectoralis* (t = 2.07, d.f. = 25.90, *P* = 0.179) as well as dragonfly-lake specialist *L. dubia* (t = 1.40, d.f. = 2 2.07, *P* = 0.540) comparing individuals from fish lakes with individuals from dragonfly lakes (Fig. 3b).

There was no significant difference in branchial chamber size comparing larvae from fish lakes with larvae from dragonfly lakes in *L. albifrons* (t = 0.67, d.f. = 39.55, *P* = 0.941), *L. pectoralis* (t = 0.50, d.f. = 29.19, *P* = 0.980) and *L. dubia* (t = 0.98, d.f. = 35.58, *P* = 0.804) (Fig. 4b).

Neither the dragonfly-lake specialist *L. dubia* (t = 2. 17, d.f. = 15.50, *P* = 0.173) nor the fish-lake specialist *L. albifrons* (t = 2.03, d.f. = 14.70, *P* = 0.224) and *L. pectoralis* (t = 1.12, d.f. = 15.61, *P* = 0.731) differed in muscle mass comparing individuals from fish lakes with individuals from dragonfly lakes (Fig. 4b).

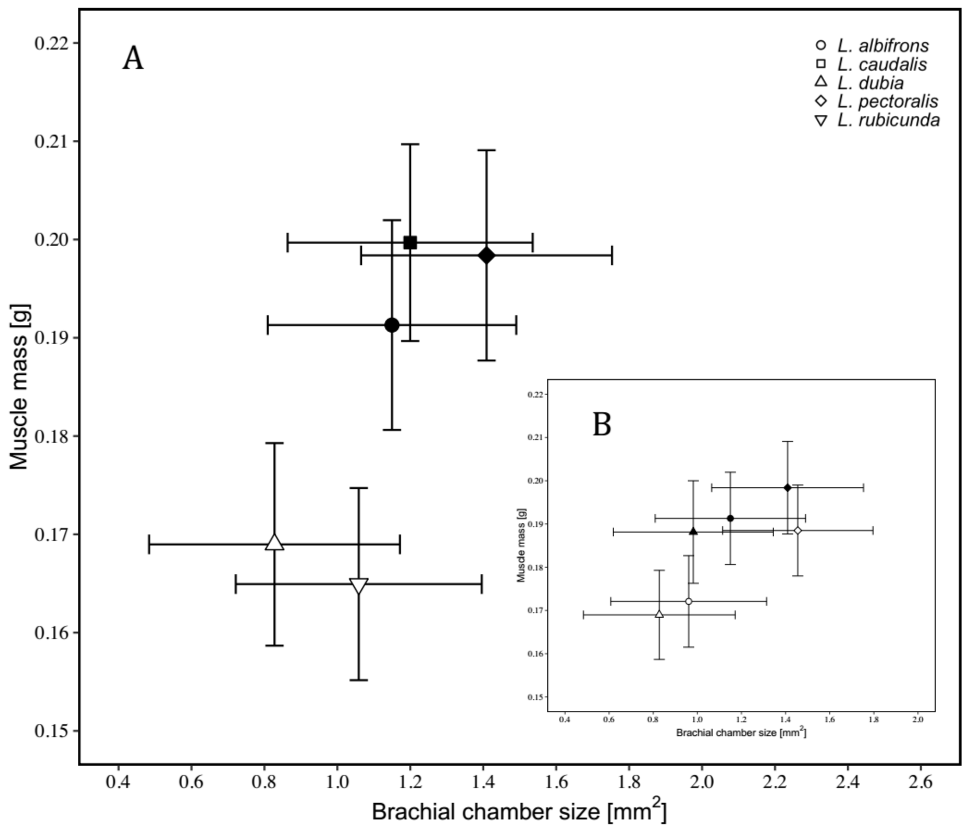


Fig. 4 Bivariate plots of mean branchial chamber size [mm^2] and muscle mass [g]. (a) Presented are means (±SE) of all 5 *Leucorrhinia* species. Black filling denotes fish-lake specialists and white filling dragonfly-lake specialists. (b) Fish-lake specialist *Leucorrhinia albifrons* and *L. pectoralis* as well as dragonfly-lake specialist *L. dubia* can also occur in low numbers in the opposite habitat (e.g. *L. dubia* in fish lakes). Presented are means (±SE) of these three species with black filling denoting individuals from fish lakes and white filling denoting individuals from dragonfly lakes.

2.5 Discussion

Predators are distributed heterogeneously along spatial and temporal axes across environments (Wellborn et al., 1996) with evidence accumulating that predation can play a major role in phenotypic divergence and trait diversification (reviewed in Vamosi, 2005; Langerhans, 2007; Schluter, 2009). Here, we show that a habitat shift-related predator change from fish lakes to dragonfly lakes among *Leucorrhinia* species and its associated reduction in selective strength resulted in a cascading alteration of the morphological machinery to facilitate evasive burst swimming. Species in dragonfly lakes evolved a narrower abdomen as well as a wider head and a longer anterior body, with the reduction in abdominal width resulting from less muscle mass and a smaller branchial chamber. Our study provides clear evidence for selection by predators to cause correlated trait changes in behavior and morphology.

Burst swimming to evade predators is a universal behavior across aquatic taxa, and differences in burst swimming are commonly mediated by changes in body shape. Faster tadpoles possess smaller bodies but larger tails (Dayton et al., 2005; Arendt, 2010), fish develop smaller heads and elongated bodies with a larger caudal region to increase speed (Langerhans et al., 2004), and faster cladocerans are less bulky (Lagergren et al., 1997). Thus, the effects of increased burst escape speed can be directly related to decreased drag and more streamlined body shape (Lagergren et al., 1997; Eroukhmanoff & Svensson, 2009; Scharnweber et al., 2013 and/or stem indirectly from, for example, increased muscle tissue (Langerhans et al., 2004; Dayton et al., 2005; Arendt, 2010). As in clades of damselfly species (McPeek, 1995), faster swimming larval *Leucorrhinia* species possess wider abdomens. McPeek (1995) hypothesized the wider abdomens to be attributed to increased muscle mass for performing powerful propulsive swimming. Even though we did not measure drag, we can relate differences in *Leucorrhinia* body shape to be mediated by increased muscle mass and increased brachial chamber size.

In the few systems known, evolutionary changes in body shape resulting from a habitat shift-related change in predation regime caused adaptations in escape speed and muscle tissue. In *Enallagma* damselflies (Brown et al., 2000) and *Leucorrhinia* (Hovmöller & Johansson, 2004), species ancestrally occurred in fish lakes and subsequently invaded dragonfly lakes, whereas in an aquatic isopod, individuals invaded fish lakes from dragonfly lakes (Eroukhmanoff & Svensson, 2009). Whereas *Enallagma* species increased burst speed and evolved a wider abdomen in response to the new invertebrate predator (McPeek, 1995; McPeek et al., 1996), *Leucorrhinia* species reduced burst speed and abdomen width in response to invertebrate predators (Mikolajewski et al., 2010). The aquatic isopod Asellus aquaticus decreased escape speed and abdomen width as an adaptation to predatory fish (Eroukhmanoff & Svensson, 2009). Thus, independent of the direction of habitat shift and change in predator threat, a higher burst escape speed is always related to a wider abdomen presumably caused by increased muscle mass (this study and indicated in McPeek, 1995 as well as Eroukhmanoff & Svensson, 2009).

Relaxed selection, by, for example, a new predator, can cause traits and their correlates to decrease (Lahti et al., 2009). Muscles are costly to produce and maintain (Zera & Denno, 1997; Marden, 2000), with relaxed selection by invertebrate predators on burst speed causing *Leucorrhinia* species to potentially save muscle-related costs. However, larval *Leucorrhinia* still need to burst escape from sit-and-wait invertebrate predators, just with less speed and consequently less muscle in comparison with pursuing predatory fish (Mikolajewski et al., 2010). Another common cost of muscle intense burst speed swimming is the performance trade-off with steady locomotion (Vanhoodydonck et al., 2001; Stephens & Wiens, 2007; Langerhans, 2009; Oufiero et al., 2011). Muscle fibre can either provide short and intensive contractions but fatigue fast or provide prolonged contraction with enhanced endurance. Such a trade-off is unlikely to play a role in *Leucorrhinia* species, as burst swimming is almost exclusively used for escape from enemies whereas walking is mainly used for non-predator-related position changes (Corbet, 1999). The branchial chamber in dragonflies also serves as the respiratory organ in dragonflies. For this purpose, larvae pump water constantly in and out of the hindgut, which is achieved by similar extrinsic and intrinsic muscles as for burst swimming (Mill & Hughes, 1966). Currently, we are not aware of a trade-off between fast burst swimming and steady muscle contraction for respiratory ventilation. However, because overall muscle work for respiration is rather low (Hughes & Mill, 1966), we do not expect such trade-off to occur.

The wider abdomen in fish-lake *Leucorrhinia* species is also partly caused by a larger branchial chamber. The branchial chamber is contracted during burst escape swimming via longitudinal and dorso-ventral abdominal muscles, ejecting water from the anus (Mill & Pickard, 1975). Jet propulsion is unique to a very few animal taxa, and within odonates, it is only present in dragonflies (Anisoptera) (Trueman, 1980). In squid, jet propulsion speed is positively correlated with mantle cavity size, and the volume of water to be ejected is correlated with the muscle strength (Trueman, 1980; Wells & Odor, 1991). A similar pattern is likely to hold for larval *Leucorrhinia*. However, even though we found an association between branchial chamber size and muscle mass, we did not measure changes in per capita muscle strength. Muscle mass and strength could interact in providing fish-lake species with a faster escape speed. Further, aperture size affects burst swimming in squid (Staaf et al., 2014). Dragonfly larvae can actively control the size of their anal valve (Pickard & Mill, 1974), but no studies so far measured the impact of the aperture size on burst swimming in larval dragonflies.

We predicted that the interspecific differences in intercorrelated trait differences should be mirrored in intraspecific differences among populations. The reason for this prediction is that new environments should select for new trait values among populations within species, which ultimately might result in speciation (Coyne & Orr, 2004; Vamosi, 2005; Nosil, 2012). Such initial differences between populations within a species might be genetically fixed or caused by phenotypic plasticity (Coyne & Orr, 2004; Vamosi, 2005; Nosil, 2012). Dragonfly-lake specialist *L. dubia* is known to be phenotypically plastic in its larval behavioral activity (Mikolajewski & Johansson, 2004) and defensive abdominal spine length (Johansson & Samuelsson, 1994; Arnqvist & Johansson, 1998). Further, differences stemming from either plasticity or genetic differentiation, comparing fish lakes and dragonfly lakes, have been identified in burst escape speed and arginine kinase activity (Mikolajewski et al., 2010) as well as in patterns of morphological integration (Mikolajewski et al., 2015b). Higher burst speed in larval *L. dubia* from fish lakes (Mikolajewski et al., 2010) is not achieved by increased muscle mass and increased branchial chamber size (this study). Thus, we assume a differences in arginine kinase activity for intense muscle exercise (Mikolajewski et al., 2010) to mediate intraspecific differences in burst speed, rather than a combination of increased muscle mass, branchial size and increased enzyme activity as can be seen interspecifically (Mikolajewski et al., 2010 and this study). This would also explain why larval *L. dubia* from fish lakes express increase burst escape speed and arginine kinase activity in comparison with individuals from dragonfly lakes, but do not match burst speeds of fish-lake specialists, *L. caudalis*, *L. albifrons* and *L. pectoralis* (Mikolajewski et al., 2010). Arginine kinase has been shown to be under strong selection (Kemppainen et al., 2011) including by predation (Strobbe et al., 2010). Our data support finding in another odonate genus showing that intraspecific differences in swimming speed to be exclusively mediated by changes in arginine kinase activity (Strobbe et al., 2010), whereas interspecific differences are mediated by morphological adaptation and increase in enzyme activity (McPeek, 1995, 1999).

Fish-lake specialists have been shown to differentiate between fish lakes and dragonfly lakes via phenotypically plastic behavior for *L. albifrons* (Mikolajewski & Johansson, 2004) and via plasticity or genetic differentiation in morphological abdominal integration for *L. albifrons* and *L. pectoralis* (Mikolajewski et al., 2015a, b). No data are yet available about burst speed differences within these two species comparing fish and dragonfly lakes. Additional common garden experiments are needed to understand the cause of any intraspecific differences in this genus. Local adaptations via genetic differentiation have been reported in damselflies (Shama et al., 2011; Gosden et al., 2015). However, we hypothesize intraspecific differences in all *Leucorrhinia* species to stem from phenotypic plasticity rather than genetic differentiation, because dragonflies (Corbet, 1999), including *Leucorrhinia* species (Pajunen, 1962; McCauley, 2006), disperse widely. Assuming intraspecific patterns to be plastic in the three *Leucorrhinia* species, this might have implications for understanding processes of diversification (Pfennig et al., 2010). Phenotypic plasticity causing intraspecific divergence to mirror species differences is expected to indicate a role or phenotypic plasticity in adaptation to new environments via changes of the reaction norms (Pfennig et al., 2010). When an organism encounters a new environment, phenotypic plasticity might allow it to survive and establish in the new environment until adaptive genetic change has occurred (West-Eberhard, 2005). Hence, a trait that was originally plastic changes its plasticity or becomes genetically fixed at a new trait value. This process is referred to as genetic accommodation and genetic assimilation, respectively (Crispo, 2007).

Here, we show that relaxed selection on burst escape speed, a common behavioral feature to escape predation, caused dramatic changes in intercorrelated morphological traits. To fully understand phenotypic evolution in response to changes in the selective regime, we recommend focusing on multiple rather than single traits to develop an integrated view about evolutionary trait changes in complex organisms and the interactions of behavioral and morphological traits.

2.6 Acknowledgments

We like to thank Axel Conrad, Katrin Mikolajewski and Linda Rüsen for help in the field. Jens Rolff, Paul R. Johnston and two anonymous reviewers gave very valuable comments on an earlier draft of this part.

Chapter III

Predator shift related adaptive changes in larval growth and digestive physiology

3.1 Abstract

Species are distributed along predator gradients with prey susceptibility being determined by preys' ability to trade off growth against predation risk. The foraging-mediated growth/predation risk trade-off is well established with increased foraging accelerating growth but also increase predator induced mortality. Adaptations in digestive physiology have been identified to modify partly the relationship of foraging and growth, making it likely that digestive physiology plays a major role in determining species ability to occur along a habitat gradient. However, studies exploring the impact of digestive physiology on growth in prey are still scarce. Larvae of the dragonfly genus *Leucorrhinia* segregate between lakes either being dominated by predatory fish (fish lakes) or predatory invertebrates (dragonfly lakes). Both predator types differ dramatically in their hunting style like searching and pursuing mode. Whereas fish-lake and dragonfly-lake *Leucorrhinia* did not differ in growth rate, we show that difference in predation regime caused *Leucorrhinia* species in fish lakes and dragonfly lakes to evolve different pathways of digestive physiology to achieve similar growth rate. Because fish-lake species expressed a higher metabolic rate than dragonfly-lake species, we assume energy to be differently allocated and used for metabolic demands between species of both predator environments. Further, growth rate but not digestive physiology was plastic in response to the presence of predators. Our results highlight the impact of digestive physiology in shaping the foraging-mediated growth/predation risk trade-off, with digestive physiology representing key variables shaping the occurrence along habitat gradients.

Key words: environmental gradient; foraging-mediated growth/predation risk; growth rate; predation; phenotypic plasticity.

3.2 Introduction

Predation is known to be a strong selective force mediating prey species phenotype as well as occurrence and abundance (Kerfoot & Sih, 1987; Wellborn et al., 1996; Vamosi, 2005; Schluter, 2009; Stroud & Losos, 2016). In response, prey evolved a multitude of defenses to avoid and repel predators (reviewed in Edmunds, 1974). Because predator species do not occur universally but segregate among different environments, this results in well described predator gradients with prey species occurrences and phenotypic appearance resembling the contemporary predation regime (e.g. Laurila et al., 2004; Stoks & McPeek, 2006; Runemark et al., 2014). As one example, the freshwater habitat gradient includes a well described change in predation regime with permanent lakes being dominated by either large invertebrate predators like dragonfly and/or beetle larvae (dragonfly lakes) or predatory fish species (fish lakes) (McPeek, 1990b; Wellborn et al., 1996). Thereby, differences in prey susceptibility and life history constraints to distinct predator species mainly determine the distribution of prey species along this habitat gradient (e.g. McPeek, 1990a; Stoks & McPeek, 2003; Mikolajewski et al., 2006; Swaegers et al., 2017).

Prey susceptibility and life history constraints are mainly mediated by preys' ability to trade-off growth and predation risk (Sih, 1987; Munch & Conover, 2003; Laurila et al., 2006). Prey species contrast vastly in their growth rates (Dmitriew, 2011) with strong growth rate difference even being apparent among closely related species (e.g. Anholt et al., 2000; McPeek et al., 2001; Mikolajewski et al., 2015a). Distinct predation regimes are considered to play a major role in selecting for predator-specific growth rates (Benard, 2004), with predation representing an important agent for growth rate diversification among closely related species (Stoks & McPeek, 2003; Schmidt & Van Buskirk, 2005; Stoks & McPeek, 2006). Behavior is a key trait balancing the foraging-mediated growth/predation risk trade-off. Briefly, prey growth rate as well as mortality is mediated by behavior with for instance higher foraging resulting in accelerated growth but also in more exposure to predators (Werner & Anholt, 1993). Whereas the foraging-mediated growth/predation risk trade-off is well established, it has been recognised that behavioral differences do not fully explain variation in growth rate among prey species (Anholt et al., 2000; McPeek et al., 2001; Suhling et al., 2005; Tigreros et al., 2018). Apparently, digestive physiology has been identified to partly decouple foraging induced mortality from growth, with growth- and conversion efficiency increasing growth independently of foraging (Stoks & McPeek, 2003, 2006; Allen et al., 2016). However, few studies have unravelled the impact of digestive physiology on growth differences among closely related species (but see McPeek et al., 2001; Stoks & McPeek, 2003).

Here we study the role of growth and digestive physiology in determining prey species turnover among a predator freshwater gradient, specifically between fish- and dragonfly lakes. We concentrate on larval of the closely related species within the dragonfly genus (Odonata: Anisoptera). Species of this genus separate among fish lakes and dragonfly lakes, with fish lakes representing the ancestral habitat (Hovmöller & Johansson, 2004). A phylogenetic study indicated that habitat shift from fish lakes to dragonfly lakes occurred twice independently (Hovmöller & Johansson, 2004), with dragonfly-lake species having had to adapt in response to the altered selective strength by changes in morphology (Mikolajewski & Johansson, 2004; Johansson & Mikolajewski, 2008; Mikolajewski et al., 2016), physiology (Mikolajewski et al., 2010) and behavior (Mikolajewski & Johansson, 2004; Mikolajewski et al., 2010). All those adaptations deal with immediate impact of predator attack by increasing survival. Less well studied is how species deal with the interaction among perceived risk of predation, foraging behavior and growth rate. Most recently, Jiang and Mikolajewski (2018) showed that dragonfly-lake species also evolved higher foraging than fish-lake species in response to a change in selective pressure. Higher foraging of dragonfly-lake species might accelerate growth rate via increased food intake. Thus, (a) we expected growth rate will be higher in dragonfly-lake species in comparison to fish-lake species. Because the expected lower foraging mediated growth rate in fish-lake species would result into prolonged development and/or smaller body size, we expected (b) fish-lake species to compensate their growth rate partly by an increase in digestive physiology. This is because prolonged development as well as reduced body size have profound negative fitness consequences for most insects (Blanckenhorn, 2000; Koons et al., 2008; Dmitriew, 2011) including odonates (Sokolovska et al., 2000). This could also result into similar overall growth rates comparing dragonfly-lake and fish-lake species. Last, we tested if dragonfly- and fish lakes species differed in their response to both predator regimes. Prey often respond plastically in behavior and physiology to varying predators (McPeek, 1990a; Richardson, 2001; Stoks & McPeek, 2003). However, it has been shown that prey might only be able to recognise their native predators (Stoks et al., 2003). Thus, we expected dragonfly-lake species only respond to invertebrate predators and fish-lake species to only respond to predatory fish physiologically.

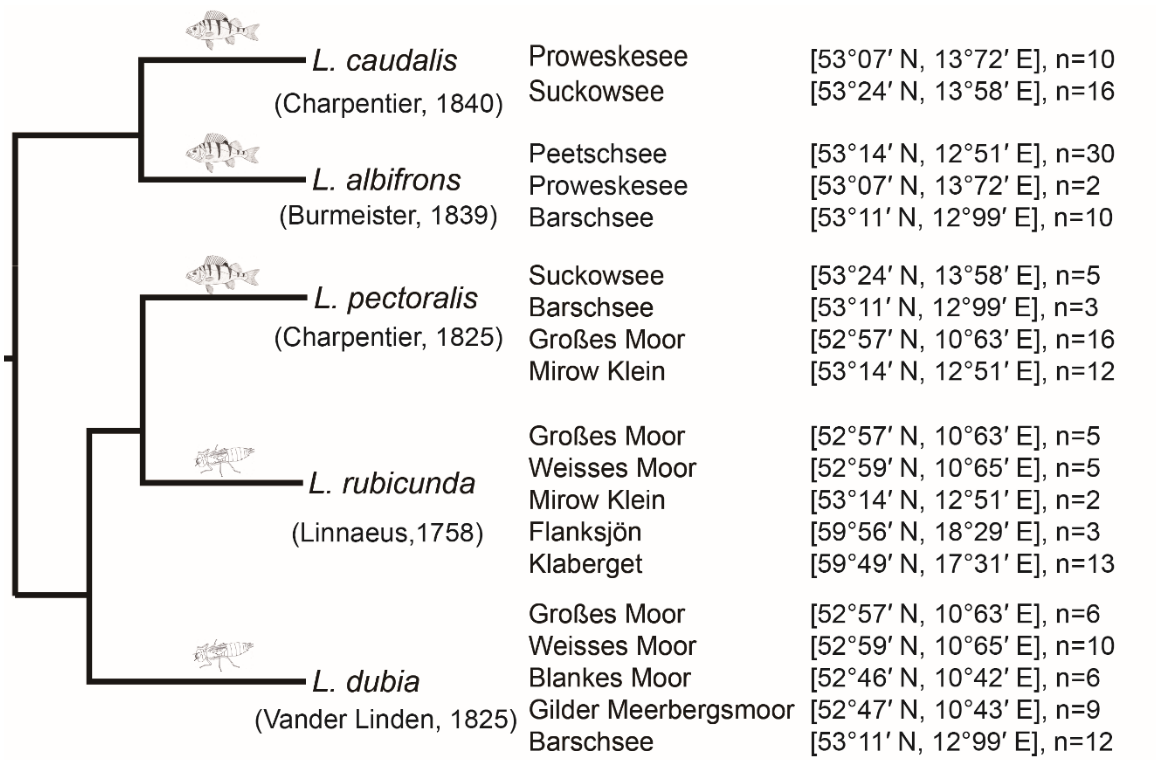


Fig. 1. Sampling locations with coordinates and phylogeny of the five used European *Leucorrhinia*-species (adapted from Hovmöller and Johansson, 2004). Predatory fish and predatory dragonfly illustrations indicate fish-lake and dragonfly-lake species. Sample size (n) are given for each species.

3.3 Materials and methods

3.3.1 Species and maintenance

Here we concentrate on the five European *Leucorrhinia*-species, with *Leucorrhinia albifrons*, *L. caudalis* and *L. pectoralis* being classified as fish-lake species, whereas *L. dubia* and *L. rubicunda* represent dragonfly-lake species (Hovmöller & Johansson, 2004;

Petrin et al., 2010). Last instar larvae of all five *Leucorrhinia*-species were collected during March and April 2016 from a minimum of two locations (Fig. 1). In all larvae we measured head width as a proxy for body size (Benke, 1970). Larvae were kept individually in opaque 80ml vials (Ø 4.5cm) filled up for 6.0cm with aged tap water. We maintained larvae in controlled temperature incubators at 10 ℃ with an 11:13 day/night light regime (corresponding with natural conditions in March) until the start of the experiment. We fed larvae *ad libitum* twice a week with alive blood worms (*Chironomus* spec.).

Perch (*Perca fluviatilis* (Linnaeus 1758)) and last instar dragonfly larvae *Aeshna cyanea* (Müller, 1764) were used as predatory fish and large invertebrate predators, respectively. Both predator species are well known to cause severe predation threat on larval *Leucorrhinia* (Mikolajewski & Johansson, 2004; Mikolajewski & Rolff, 2004; Mikolajewski et al., 2006). We kept four perch (body length ~15cm) in two 240L aquarium (120cm × 40cm, height 50cm). Perch were feed *ad libitum* every second day with alive blood worms. Because larval aeshnids are strongly cannibalistic, we kept fourteen larvae in separate opaque plastic containers (7cm × 4.5cm, height 10cm), with containers floating in two 120L aquarium (30cm × 20cm, height 20cm). Each container was provided with a piece of plastic gauze (5cm × 3.5cm) for perching and was pierced with 20 holes (Ø 1mm) to ensure water exchange between containers and aquaria. Each larval aeshnid was fed *ad libitum* with alive blood worms every second day. For the control treatment we arranged two 120L aquaria with aged tap water only.

3.3.2 Growth rate experiment

We carried out a 7-day predator-exposing experiment to estimate growth rate and digestive parameters in larval *Leucorrhinia*. The experiment was done in five blocks during 8th Apr. to 10th May. Two days before the experiment, we fed *Leucorrhinia* larvae *ad libitum* blood worms, to align their physiological conditions. Experiments took place in a constant temperature room at 19 ℃ and a light regime of 11:13 day:night cycle. Twenty-four hours before measurements larval *Leucorrhinia* were transferred into the constant temperature room for larvae to adjust to experimental conditions and to empty their guts. The following day, larval *Leucorrhinia* were allocated randomly into three treatments: (1) control, (2) predatory dragonfly predator, and (3) predatory fish by using water from the control, predatory fish, and predatory dragonfly aquaria (each treatment had two aquaria). Water from the two corresponding tanks for each treatment was mixed beforehand. Wet weight of each larval *Leucorrhinia* was determined with an electronic balance Precisa, XR205SM-DR (Th. Geyer, Switzerland) to the nearest 1mg and larvae were placed individually into opaque containers (7.5cm×4.5cm, height: 10cm) with 4cm high water corresponding with the treatments (see above). Each larva was feed for six days with 5-10 blood worms (*Chironomus* spec.) daily. The food ration was adjusted for each larva so that at least one blood worm was left the next day to ensure *ad libitum* feeding. Blood worms used as food were selected for equal size. For each day we estimated the amount of consumed blood worms of each *Leucorrhinia* larva by drying (drying oven at 60℃ for 24h), and we weighed the same amount of blood worms that was fed to the nearest 1mg. Further, each day leftover food and faeces from each individual larva was collected. On day seven, larvae were not fed but given time to empty their guts, with faeces being collected and the final weight of each larva was measured on the nearest 1mg. Leftover food and collected faeces of each larva from the former days were dried (see above) and were weighed to the nearest 1mg.

The following dependent growth rate and digestive parameters were calculated closely following Stoks et al. (2005). (a) Individual larval growth rate was qualified as [ln(final wet weight) - ln(initial wet weight)]/7d (Stoks et al. 2012). As a measure for the amount of food consumed by each larva we calculated (b) the total amount of ingested food [(total mass supplied food) – (total mass uneaten food)]. (c) Growth efficiency [(body mass increase)/(total mass ingested food)] was calculated to estimate how much of the total amount of ingested food translated into body mass. For further detail, we calculated two parameters describing how the assimilated food (total mass ingested food – total mass faeces) was used physiologically. (d) Assimilation efficiency (total mass assimilated food)/(total mass ingested food) was calculated to estimated how much of eaten food was assimilated for growth and metabolism whereas (e) conversion efficiency (total body mass increase)/(total mass assimilated food) shows how much of the assimilated food is translated into body mass. Last, to develop an idea about the metabolic rate of each larva, we recorded (f) oxygen consumption as a proxy for basal metabolic rate (Stoks et al., 2006; De Block et al., 2008). Measures for oxygen consumption were taken on day seven of the experiment with each larva being transferred into a separate lidded 80 ml vial. For each treatment, vials were filled with treatment corresponding oxygen-saturated water (same as in the growth rate experiment). The oxygen content in the vial was measured before and after 24 h with a HACH HQd Oxygen analyser (HACH, Germany). To correct for the background changes of different water medium in treatments, we measured five additional replicates which contained only water mediums without larva for each treatment. The oxygen consumption was calculated as [(total final oxygen content) – (initial oxygen content)-(background correction)]/24h (Stoks et al., 2006).

3.3.3 Data analyses

Because we did not detect a phylogenetic signal (K_{mult}, Package “geomorph” (Adams & Otárola-Castillo, 2013)) in our data (see Supplementary material Table S1) for any of the six dependent variables (see above), we did not apply phylogenetic data analyses. Instead fitted separate linear mixed effect models (package lme4, (Bates et al., 2016)) for the six dependent growth and digestive variables (see above). As explanatory variables we used species and treatment fully factorial, with head width as an additional additive independent factor to control for body size differences among *Leucorrhinia* larvae. Further, we nested sample location within species as random effect, because not all species occurred in every location. Because the experiment took place in three blocks, we added block as a random effect to our models. To achieve normal distribution in residual error structure, we transformed the total amount of ingested food, the conversion efficiency and oxygen consumption with log10(x), log(x) and log(x+1), respectively.

Within the six fitted linear-mixed effect models we applied a set a priori linear contrasts (package emmeans, (Lenth, 2018)). First, we tested if (1) fish-lake species differed from dragonfly-lake species (*L. albifrons*/*L. caudalis*/*L. pectoralis* vs. *L. dubia*/*L. rubicunda*), Second, we tested if fish-lake species (2) responded to predatory fish (control tretament in *L. albifrons*/*L. caudalis*/*L. pectoralis* vs. fish treatment in *L. albifrons*/*L. caudalis*/*L. pectoralis*) and (3) responded to predatory dragonflies (control tretament in *L. albifrons*/*L. caudalis*/*L. pectoralis* vs. dragonlfy treatment in *L. albifrons*/*L. caudalis*/*L. pectoralis*). Third, we tested if dragonfly-lake species (4) responded to predatory dragonfly (control treatment in *L. dubia*/*L. rubicunda* vs. dragonfly treatment in *L. dubia*/*L.*

rubicunda) and (5) responded to predatory fish (control treatment in *L. dubia*/*L. rubicunda* vs. fish treatment in *L. dubia*/*L. rubicunda*).

3.4 Results

3.4.1 Growth rate and digestive physiology

Growth rate did not differ between fish-lake and dragonfly-lake species (Table 1, contrast 1 (a); Fig. 2A). However, fish-lake species ingested significantly more food (Table 1, contrast 1 (b); Fig. 2B) but expressed significantly lower growth efficiency (Table 1, contrast 1 (c); Fig. 2C) than dragonfly-lake species. Further, fish-lake species expressed a significantly higher assimilation efficiency (Table 1, contrast 1 (d); Fig. 2D) but significantly lower conversion efficiency (Table 1, contrast 1 (e); Fig. 2E) than dragonfly-lake species. Fish-lake species have significantly higher oxygen consumption than dragonfly-lake species (Table 1, contrast 1 (f); Fig. 2F). Please note, even though digestive traits differed significantly between fish-lake and dragonfly-lake species, differences in ingested food, growth efficiency, conversion efficiency and oxygen consumption were mainly caused by significant differences of *L. dubia* from fish-lake species whereas *L. rubicunda* did not differ significantly from fish-lake species (Supplementary material, Table S2).

3.4.2 Plastic response in growth rate and digestive physiology

Fish-lake species significantly increase growth rate in response to the presence of predatory dragonflies (Table 1 contrast 3 (a); Fig. 2A), but not change growth rate in the presence of predatory fish (Table 1 contrast 2 (a); Fig. 2A). None of the digestive parameters differed significantly in response of the presence of predatory fish (Table 1 contrast 2 (b-f); Fig. 2B-F) not to the presence of predatory dragonflies (Table 1 contrast 3 (b-f); Fig. 2B-F).

Dragonfly-lake species significantly accelerated growth rate in response to the presence of predatory dragonflies (Table 1 contrast 4 (a); Fig. 2A) as well as in the presence of predatory fish (Table 1 contrast 5 (a); Fig. 2A). Whereas none of the digestive parameters differed significantly in response of the presence of predatory dragonflies

(Table 1 contrast 4 (b-f); Fig. 2B-F), larvae significantly increased the amount of ingested food and the assimilation efficiency in response to predatory fish (Table 1 contrast 5 (b & d); Fig. 2B & D). No other significant differences were found (Table 1 contrast 5 (c, e, f); Fig. 2C, E, F).

Table 1. A priori-hypotheses based linear contrasts (see Materials and methods) for differences in growth rate and five digestive traits between (A) fish-lake and dragonfly-lake species, and between (B) different predator treatments (no predator/predatory fish/predatory dragonfly) for fish-lake and dragonfly-lake species. Presented are t-values with adjusted *df* in brackets.

Contrasts	Growth rate (a)	Ingested food (b)	Growth efficiency (c)	Assimilation efficiency (d)	Conversion efficiency (e)	Oxygen consumption (f)
A) Between groups difference						
1. Linear contrasts	$0.43_{(62.68)}$	$2.31_{(39.5)}$*	$2.55_{(65.08)}$*	$3.22_{(37.31)}$*	$3.00_{(66.3)}$*	$2.11_{(37.55)}$ *
B) Group responses to different predators (treatment contrasts)						
<u>Fish-lake species</u>						
2. fish vs. control	$1.71_{(169.46)}$†	$1.06_{(165.5)}$	$1.17_{(168.30)}$	$0.56_{(179.26)}$	$0.84_{(171.51)}$	$1.\ 40_{(179.88)}$
3. dragonfly vs. control	$2.25_{(169.16)}$*	$0.93_{(166.9)}$	$1.87_{(169.21)}$†	$1.33_{(178.69)}$	$1.55_{(172.69)}$	$1.68_{(179.64)}$†
<u>Dragonfly-lake species</u>						
4. dragonfly vs. control	$2.17_{(179.20)}$*	$1.56_{(171.41)}$	$1.35_{(177.93)}$	$1.86_{(185.21)}$†	$1.35_{(179.43)}$	$1.02_{(186.71)}$
5. fish vs. control	$2.43_{(177.74)}$*	$2.24_{(169.83)}$*	$0.81_{(176.50)}$	$2.46_{(185.32)}$*	$0.96_{(179.40)}$	$0.84_{(186.62)}$

Note: Significance noted: * $0.05>P$, † $0.1>P>0.05$

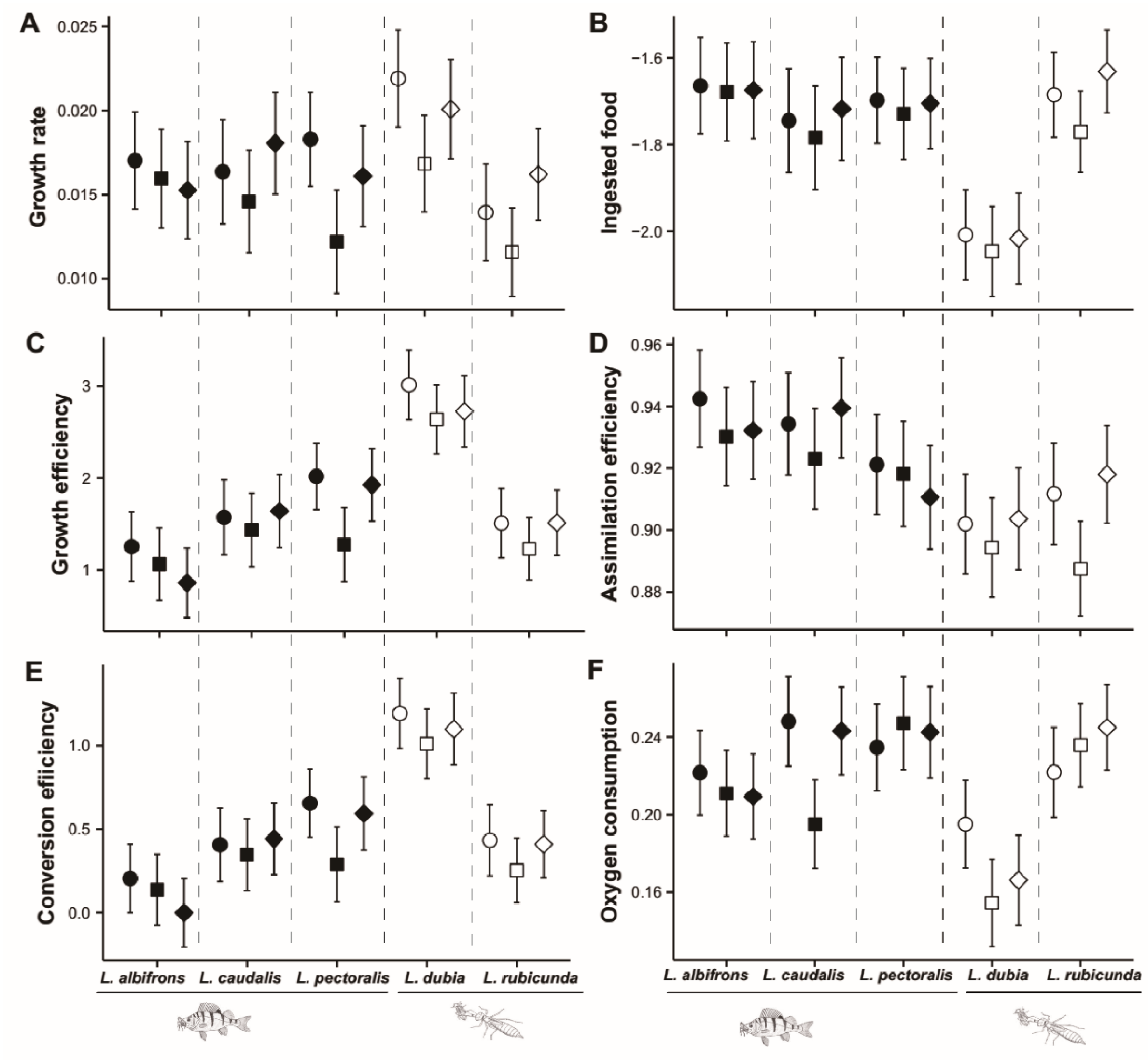


Fig. 2 Emmeans ±1SE from linear mixed effect models (see material and methods) for species and treatments comparisons of (A) growth rate, (B) the amount of ingested food, (C) assimilation efficiency, and (D) conversion efficiency, (E) growth efficiency and (F) oxygen consumption in five European *Leucorrhinia* species. Filled symbols indicate fish-lake species whereas non-filled symbols indicate dragonfly-lake species. Shapes indicate treatments (●: dragonfly; ■: control; ◆: fish).

3.5 Discussion

Because growth rate mediates body size directly, it is a key trait determining an organism's fitness (Peckarsky et al., 2001; Dmitriew, 2011). Growth rate also narrows species occurrence along habitat gradients like different predator regimes, with behavioral mediated growth determining prey vulnerability (Stoks & McPeek, 2003; McPeek, 2004). *Leucorrhinia*-species segregate between fish lakes and dragonfly lakes, with predatory fish being considered the more dangerous predators (Stoks & De Block, 2000). Here, we show that fish- and dragonfly-lake species do not differ in growth rate but differ in key physiological traits mediating growth rate. Consequently, physiology plays an important role in shaping growth rate, and differences in digestive traits might decouple growth partly from the impact of changed foraging behavior facing predators.

The foraging-mediated growth/predation risk trade-off is well established, with preys' vulnerability as well as preys' growth being both mediated via behavior only (Werner & Anholt, 1993; McPeek, 2004; Lind, 2005). In order to avoid predators, prey reduces foraging activities, which will usually lead to low food intake and decelerated growth (Benard, 2004; Dmitriew, 2011). Because of better encountering and detecting ability (McPeek, 1990a), predatory fish cause higher mortality in *Leucorrhinia* than predatory invertebrates (Mikolajewski et al., 2006), however note, differences in selective strength on growth rate has not yet been finally proven in our system. In order to avoid being killed by predatory fish, *Leucorrhinia*-species from fish lakes have evolved lower foraging and activity than dragonfly-lake species to avoid predation (Jiang & Mikolajewski, 2018). However, the lower foraging and activity did not result in a lower growth rate among fish-lake species but fish-lake species ingested more food as well as exhibited a higher assimilation efficiency than dragonfly-lake species (this study). Similar findings have been shown in *Enallagma*-species from fish lakes consuming more food than dragonfly-lake species (McPeek et al. 2001). Because growth rate translates directly into body size, with fitness being positively correlated to body size in most species (Brown et al., 1993) including odonates (Sokolovska et al., 2000), such physiological adaptation to increase growth prevent fish-lake species to prolong development or decrease body size. Please note that the found higher food ingestion by fish-lake species in comparison to dragonfly-lake species in our experiment is not behavioral mediated, because experimental vials were rather small impeding effects of different behavior in foraging and food intake.

Fish-lake and dragonfly-lake species also differ in how growth rate is shaped, with dragonfly-lake species having evolved higher growth efficiency and conversion efficiency than fish-lake species. In fact, species along habitat gradients have been shown before to differ in key digestive physiological traits (McPeek et al., 2001; Stoks & McPeek, 2003, 2006). Our results match findings in *Lestes*-damselflies, with species from fish lakes showing reduced growth efficiency and conversion efficiency than species from dragonfly lakes (Stoks & McPeek, 2003). Physiology seems to represent a key variable shaping the occurrence along habitat gradients via affecting the behavioral mediated growth-predation trade-off. However, species might convert different degree of the amount of ingested food into body mass by e.g. allocating it to different tissues or use it for different metabolic demands. For instance, fish-lake species expressed higher oxygen consumption than dragonfly-lake species. Higher oxygen consumption can be used as an indicator for an increased metabolic rate and usually is enhanced under environmental stress (Stoks et al., 2006; De Block et al., 2008). Prey is expected to allocated energy towards escape behaviors (e.g. “fight or flight”), resulting in an increased metabolic rate (McPeek et al., 2001). For instance, fish-lake species need to be prepared to escape from attacking predatory fish via rapid burst swimming whereas burst swimming is less important for dragonfly-lake species (Mikolajewski et al., 2010). Energy for burst escape swimming is provided via the enzyme arginine kinase, a protein that is costly to maintain (Kucharski & Maleszka, 1998). Further, elevated levels of hormones to environmental stressors like predators will increase the metabolic rate (Hawlena & Schmitz, 2010b). Because predatory fish are considered more dangerous predators than predatory invertebrates, we might expect higher physiological stress in fish-lake species than in invertebrate species and increased metabolic. However, the increase in oxygen consumption and accelerated metabolic rate also comes with severe costs like oxdative damage (Slos & Stoks, 2008; Janssens & Stoks, 2013). Because it becomes more and more apparent that physiology play a large part in determining growth (Hawlena & Schmitz, 2010a; Van Dievel et al., 2015), further studies need to unravel the detailed benefits and costs of those in shaping the growth/predation risk trade-off.

Even though we found distinct differences in digestive physiology between fish-lake and dragonfly-lake species, differences in digestive physiology between fish-lake species differed more to *L. dubia* and less to *L. rubicunda*. One possible explanation is that

L. dubia diverged early from fish-lake species than *L. rubicunda* and consequently had more time to evolve distinctive digestive traits. However, no detailed information is available about divergence time in *Leucorrhinia*-species. Secondly, further environmental differences among species might have affected the evolution of growth rate, with for instance abiotic factors impacting on the richness of food resources (Claramunt & Wahl, 2000). *L. dubia* tends to survive better in low PH ponds than the other *Leucorrhinia* species including *L. rubicunda* (Johansson & Brodin, 2003). However, food availability is reduced with high levels of acidity (Harvey & McArdle, 1987; Schell & Kerekes, 1989; Rychła et al., 2011) which might have selected for higher conversion and growth efficiency rate in *L. dubia*. Overall, our results suggest that growth rate differences among species can be caused by several interacting factors, with unique characters of one species potentially playing an important role in the evolutionary trajectories (Langerhans & DeWitt, 2004).

Prey often response plastically in growth rate and digestive physiology depending on the current environmental conditions (Beckerman et al., 2007; Stoks et al., 2012; Pujol-Buxó et al., 2016). *Leucorrhinia* species from both predation regimes increased their growth rate in the presence of predators. Increasing growth rates has been found in a number of species including odonate larvae (Slos & Stoks, 2006; Stoks et al., 2012) and fish (Johansson & Andersson, 2009). Prey will increase growth rate under the risk of predation if by growing fast prey can reach a size refuge earlier (Stoks et al., 2012). Size dependent-predation is common in predator-prey interactions (Lundvall et al., 1999; Claessen et al., 2002) and both, predatory fish and predatory invertebrates, are gape-limited (Price et al., 2015) or size-limited (Warren & Lawton, 1987). However, no such data for *Leucorrhinia*-species are available. Secondly, increasing growth rate can reduce the time being exposed to predation risk (Abrams & Rowe, 1996; McCollum & Leimberger, 1997). Because all species commonly finish development within 2 year and emergence within a rather short period of time with only little variation (Sternberg & Buchwald, 2000), we assume *Leucorrhinia*-species to shorten development in response to predators to be unlikely. In contrast, digestive traits were hardly plastic in response to predators. This is different to *Lestes*-damselflies, where digestive traits and growth rate responded plastically to the presence of predators (Stoks and McPeek 2003). Further, in *Lestes* and *Enallagma*-species, fish-lake species responded plastically in growth rate to predatory fish and predatory invertebrate whereas dragonfly-lake species only responded to predatory invertebrates (Stoks & McPeek, 2003). Our *Leucorrhinia*-species from dragonfly lakes responded in growth rate to both predator types whereas fish-lake species only responded to predatory invertebrates. Invertebrate predators are most often also present in fish-lake albeit in very low densities (McPeek, 1990b). Fish-lake *Leucorrhinia* species are well defended against predatory fish via abdominal spines (Mikolajewski & Johansson, 2004) which increased mortality with invertebrate predators (Mikolajewski et al., 2006). Therefore, it seems likely that fish-lake species only increase growth rate to outgrow predatory invertebrates rather than predatory fish, especially because increased growth rate comes with physiological costs (Blanckenhorn, 2000). In contrast, dragonfly-lake species can also occur frequently in fish lakes, albeit in very low densities (Johansson & Brodin, 2003; Mikolajewski & Johansson, 2004). Since they do not show any morphological defense against any of the two predators, they would consequently need to increase growth rate with both predators to reach a safe size refuge.

In conclusion, *Leucorrhinia* species from different predation regimes showed similar growth rate, which is likely to be achieved by different combination of foraging behavior and digestive physiological. Further, species from both predator environments reacted plastically in growth rate to the presence of predators. Behavior via the foraging-mediated growth/predation risk trade-off as well as digestive physiology determines how species are distributed along the predator type mediated habitat gradient. Consequently, understanding the evolution of key phenotypes and their performance helps us to understand how ecological communities are shaped and might alter in response to environmental change.

3.6 Acknowledgments

We like to thank J. Rolff for providing comments on an earlier version of this part. C. Manthey and A. Conrad helped sampling larvae, M. Friedrichs collected the perch and A. Conrad provided the drawings in Fig. 1.

3.7 Appendix

Table S1. Phylogenetic signal detection for all growth rate and digestive traits. K_{mult} (Adams 2014) and corresponding *P* value are shown in the table. Package "geomorph" are used here.

Traits	Growth rate (a)	Ingested food (b)	Growth efficiency (c)	Assimilation efficiency (d)	Conversion efficiency (e)	Oxygen consumption (f)
Phylogenetic signal	K_{mult}=0.20, *P*=0.72	K_{mult}=0.80, *P*=0.19	K_{mult}=0.76, *P*=0.16	K_{mult}=0.86, *P*=0.12	K_{mult}=0.83, *P*=0.12	K_{mult}=0.66, *P*=0.23

Table S2. Linear contrasts on growth rate and digestive traits of fish-lake species vs. *L. dubia* and *L. rubicunda*. Presented are t-values with adjusted *df* in brackets.

Contrasts	Growth rate (a)	Ingested food (b)	Growth efficiency (c)	Assimilation efficiency (d)	Conversion efficiency (e)	Oxygen consumption (f)
Fish-lake species vs. *L. dubia*	$1.46_{(84.29)}$	$3.80_{(51.13)}$*	$3.77_{(91.16)}$ *	$2.59_{(42.68)}$*	$4.17_{(86.87)}$ *	$3.41_{(43.34)}$*
Fish-lake species vs. *L. rubicunda*	$1.01_{(50.59)}$	$0.20_{(33.43)}$	$0.47_{(71.13)}$	$2.49_{(54.31)}$ *	$0.16_{(56.04)}$	$0.45_{(54.09)}$

Notes:* 0.05>P

Chapter IV

Predation promotes diversification in mean and variance of antipredator traits

4.1 Abstract

Predator species are separated along habitat gradients, with predation to be known to play an essential role in species traits diversification. Because predator species differ dramatically in their hunting style and mode, change of predator species will alter the mean of prey's antipredator traits as well as affect traits variance. Population trait variation has an impact on community ecology. It influences species niche width and species interactions in the food web. However, empirical studies on variance change by predation are scarce. In this study, we collected large numbers of *Leucorrhinia pectoralis* exuviates from lakes with predatory fish (fish lakes) and lakes with large invertebrate predators (dragonfly lakes) and compared their morphologic traits (spines and body size). We found that in dragonfly lakes, individuals grew shorter spines and smaller body size than individuals in fish lakes. Moreover, populations from dragonfly lakes showed smaller variance of spine length than populations from fish lakes; while populations from dragonfly lakes also had larger variance in body size than populations from fish lakes. These results indicate that trait variance as well as mean is strongly modified by different predation regimes. Studying mean and variance of traits can help to define the mode of selection forces (directional selection and stabilizing selection) in the nature. Moreover, dragonfly larvae might be a perfect organism to study phenotypic selection on quantitative traits in the wild.

Key words: dragonfly larvae; mean and variance; phenotypic selection; population diversification; predation change, skewness.

4.2 Introduction

Predators represent a major source of selection shaping preys' phenotypes (Benard, 2004; Vamosi, 2005). As a response prey has evolved a large magnitude of traits to avoid and repel predators (Edmunds, 1974; Benard, 2004; Schmitz, 2017). Because predators do not occur ubiquitously but are restricted to different habitats, prey species often segregate along predator gradients (McPeek, 1990a; Stoks & McPeek, 2003; Mikolajewski et al., 2006; Swaegers et al., 2017). Key prey antipredator traits are expected to impact the outcome of predator-prey interactions, thus, determine occurrence to different parts of the predator gradients (McPeek, 1990a; Petrin et al., 2010). Morphological defences against predators have long been in focus, because of often prominent and striking features (Bourdeau & Johansson, 2012). Thereby, spines have received much attention because they protect a large array of prey species against predatory fish (Dahl & Peckarsky, 2002; Vamosi & Schluter, 2004; Johansson & Mikolajewski, 2008). Body size is a classical trait studying predator mediated phenotypic divergence because body size is directly linked to fitness (Honěk, 1993).

A variety of studies has shown predation to cause prey population divergence in behavioral, life history, morphological and physiological attributes (Benard, 2004; Herberholz & Marquart, 2012; Davenport et al., 2014). Predators differ in hunting and pursuing features as well as densities among habitats (McPeek, 1990b; Wellborn et al., 1996). Thus, changes in selective direction and strength among different predators are the major driver of prey population divergence (Herczeg et al., 2009; Franks & Oxford, 2017). Past work on studying population divergence has mainly focused on the change in mean trait values (Violle et al., 2012), with data on changes in trait variances in response to different predators being scarce (but see Runemark et al., 2014; Garamszegi & Moller, 2017)). However, as prime target of natural selection, differences in trait variances among populations might indicate incipient speciation (Tregenza et al., 2000; Gosden et al., 2011), and inform about the relationship of phenotypic variation and fitness (Nosil & Crespi, 2006). Thus, information about combined changes in trait mean and trait variance can inform us about population dynamics as well as evolutionary dynamics.

Freshwater habitats offer a great opportunity to study trait dynamics in population divergence, because habitats typically vary in the top predator assemblage. Permanent ponds and lakes are characterized by either the occurrence of predatory fish (hereafter called fish lakes) or the occurrence of large predatory invertebrates (hereafter called dragonfly lakes) (Wellborn et al., 1996). Both predator species differ in a variety of prey detection, pursuing and capture features, resulting in differential selection on a set of adaptations in prey (Benard, 2004). Thus, selection by both predator regimes is responsible for differential occurrence of prey species along the freshwater habitat gradient (Sih et al., 1985; Stroud & Losos, 2016) as well as can cause population divergence within prey species (Dahl & Peckarsky, 2002; Blumstein & Daniel, 2005; Magalhaes et al., 2016).

Selection on antipredator traits by predatory fish and predatory invertebrates can be, however, context dependent. In spines, predatory fish select for long abdominal spines (Johansson & Mikolajewski, 2008), whereas antagonistic selection by predatory invertebrates result in reduced spine length (Mikolajewski et al., 2006). Such directional selection would result in overall different means between fish-lake and dragonfly-lake populations (Johansson, 2002). However, patterns in trait variance might look more complex. Defensive spines are likely to be costly to produce (Mikolajewski & Johansson, 2004; Flenner et al., 2009). Thus, predatory fish select for long spines as a defence, large habitat complexity providing shelter will also allow individuals with short spines to survive (Henrikson, 1993), saving production costs. In contrast, individuals in dragonfly lakes will tend to shorten spines because of selection against spines by invertebrate predators and because of saving production cost of spines. This might result in stronger stabilizing selection for short spine length in dragonfly lake populations than for long spine length in fish-lake populations, resulting in lower variance in populations from dragonfly lakes than in fish lakes.

Body size represents a key phenotypic trait affecting most aspects of an individual life and relationship with other organisms (Blanckenhorn, 2000; Dmitriew, 2011). Body size is under considerable selection by predators (Warren & Lawton, 1987; Ziemba et al., 2000; Schmidt & Van Buskirk, 2005) and usually scales with growth rate (Peters & Peters, 1986; Relyea, 2001a; Peacor et al., 2007). In cases where predation risk is size-dependent, shifts in mean body size between predators can be expected if predator types favour different prey sizes (Price et al., 2015). Variation in body size can increase, via e.g. predator mediated behavioral changes differing between predator types, because of scaling effects of growth rate with body size (Uchmański, 1985; Peacor et al., 2007). For instance, variation in body size is predicted to increase, if larger individuals, that are safe from predation, will proportionally grow more than smaller, more vulnerable individuals (Eklöv & Werner, 2000). In contrast, we can also expect shifts in mean body size, if predators cause different selective strength on foraging behavior (Reznick, 1982; Ercit, 2016). In this case body size variation is predicted to be lower under stronger reduction in predator mediated behavioral changes, because of decreased food intake mediating reduced growth and decreased individual variation (Ziemba et al., 2000; Peacor et al., 2007).

Here we study mean and variance trait changes in defensive spine and body size among populations of larval *Leucorrhinia pectoralis* (Charpentier, 1825). Species of the genus *Leucorrhinia* separate strongly between fish lakes and dragonfly lakes (Hovmöller & Johansson, 2004; Petrin et al., 2010), whereas some species including *L. pectoralis* can occur in both predator-dominated habitats (Johansson & Brodin, 2003; Petrin et al., 2010; Mikolajewski et al., 2016). Thereby, intraspecific trait divergence is showing similar directions and magnitude of traits expression like interspecific differences (Mikolajewski et al., 2010; Mikolajewski et al., 2016). Larval *Leucorrhinia* in fish lakes express dominant abdominal spine that are effective against predatory fish (Johansson & Mikolajewski, 2008), whereas antagonistic selection by predatory invertebrates results in reduction of spine length (Mikolajewski et al., 2006). Based on these patterns, we hypothesise (i) fish-lake population to possess longer abdominal spines than dragonfly-lake populations. Further, because of the above described relationship of different costs and benefits in spine length between fish-lake and dragonfly-lake populations, we hypothesise (ii) fish-lake population to exhibit larger variation in spine length than dragonfly-lake populations. Less well studied is the relationship of body size in relation to both predation regimes. No data are available if size selectivity differ between both predators, however, data from other species indicate that prey might become saver with increasing body size in both predators (Mikolajewski & Johansson, 2004; Stoks et al., 2012). There is evidence for larval *Leucorrhinia* of dragonfly lakes to express higher foraging behavior (Jiang &

Mikolajewski, 2018) but growth rates did not differ because of compensatory physiological mechanisms (Bin et al. submitted). Consequently, we hypothesise (iii) no differences in mean body size between fish-lake populations and dragonfly-populations. Further, because growth rate and food intake were similar in experimental trials among larval *Leucorrhinia* from fish lakes and dragonfly lakes, we hypothesis (iv) no difference in body size variance among fish-lake and dragonfly-lake populations.

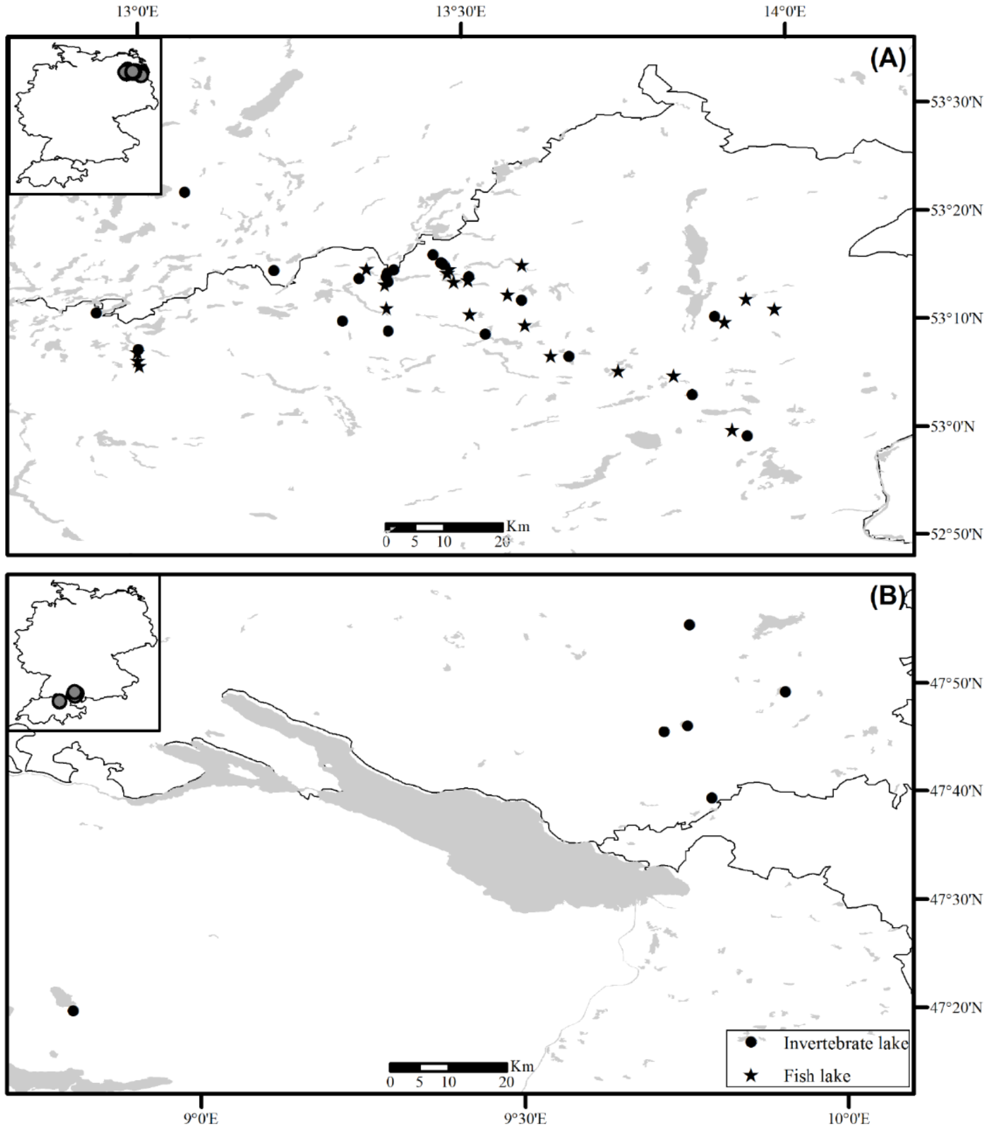


Fig. 1 Sampling locations and information of predation regime in each sampling site. Filled circles represent lakes with only predatory invertebrate (dragonfly lakes), and filled stars mean lakes with predatory fish (fish lakes).

4.3 Materials and method

4.3.1 Sample collection

We collected exuviae of *L. pectoralis* from 46 permanent lakes and ponds across Germany and Switzerland (Fig. 1). Exuviae were collected on emergent vegetation along the shoreline. We selected shorelines due to our previous experience representing typical odonate emergence habitats. During 1990 to 2015, exuviae were collected from mid to end of May which represent the main emergence period of *L. pectoralis* (Sternberg & Buchwald, 1999). Exuviae were stored dry per collection site and year at room temperature; sample size per collection site and year is given in Table S1 (Appendix).

Collection sites differed in the occurrence of predators, with predatory fish being either present or absent. Presence of predatory fish was determined by fishing or data provided by local fisherman, angling societies and provincial environmental authorities. Common species being present were crucian carp (*Carassius carassius*, Linnaeus, 1758), pike (*Esox Lucius,* Linnaeus, 1758), perch (*Perca fluviatilis*, Linnaeus, 1758), common roach (*Rutilus rutilus*, Linnaeus, 1758) and common rudd (*Scardinius erythrophthalmus*, Linnaeus, 1758). In site with no predatory fish, common large invertebrate predators detected by net sampling, collection of exuviae and dead individuals were larvae of the dragonfly family Aeshnidae, larvae of the beetle genus Dytiscus and species of the backswimmer genus Notonecta.

4.3.2 Spine length and body size measurements

We took digital photos of each exuvia from dorsal, ventral and lateral sides using an Olympus digital microscope SZX16 (Hamburg, Germany). Measurements of abdominal spine length and body size were obtained from photos using the free software ImageJ 1.50g (National Institutes of Health, USA 2016).

Abdominal spine length: Length of dorsal posterior spines at abdominal segment 5-8 and lateral spines at abdominal segments 8 and 9 were measured from the base to the tip of the spines (Johansson & Samuelsson, 1994). We did not include dorsal anterior abdominal spines because those are covered by wing pads and are not under selection by predators (Petrin et al., 2010).

Body size: Head width represents the most common body size surrogate in odonate larvae (Benke, 1970). However, head width cannot be measured in exuviae, because of the split open head capsule from emergence. Therefore, we used labium length as the total length of the prementum (Dudgeon, 1989) and length of the pro-, meso- and meta- femur and tibia (Falck & Johansson, 2000; Petrin et al., 2010) as surrogates for body size. Measurements for femur and tibia length was only used from the right side.

4.3.3 Statistical analyses

Because of the high multicollinearity among our abdominal spines and body size measurements, we first applied principal component analyses (PCA, package “psych”, (Revelle, 2016)) on the full data set using covariance matrix to reduce the number of variables. PCA extracted two principal components (PC) explaining 74% of the full variance, with body size surrogates loading high on PC1 (PC Body size, Table 1). PC2 captured variance from all abdominal spine measures (PC Spines, Table 1). The two extracted principal components were used in any subsequent analyses.

To evaluate differences between fish-lake and dragonfly-lake populations in trait means of abdominal spine length (PC Spines) and body size (PC Body size), we applied mixed effect models (packages “spaMM”, (Rousset, 2017)) using predator regime (fish-lake vs. dragonfly-lake) as a fixed effect and sampling years as well as sampling locations nested in predator regime as random effects. In the model, trait variance heteroscedasticity in different predation regimes was allowed.

To test for differences in trait variance of abdominal spine length (PC Spines) and body size (PC Body size) between dragonfly-lake populations and fish-lake populations we first extracted the residuals from a linear mixed effect model with sampling year only as random effect, to remove variance related to sampling years. Extracted residuals were used in following Levene’s tests for total variance differences between fish-lake and dragonfly lake populations as well as for comparing between-population variances among fish-lake and dragonfly-lake populations. In order to test whether total variance is greater among fish-lake than among dragonfly-lake populations, we applied Levene’s test across fish-lake populations and dragonfly-lake populations. To test whether between-population variances show the same pattern as total variances, we firstly calculated the mean of PC spines and PC body size of each population; secondly, we applied Levene's test on those mean estimates to compare between-population variance across fish-lake and dragonfly-lake populations. In order to visualize variances components between fish-lake and dragonfly-lake populations, we decomposed total variances into between- and within-population variances.

In addition to mean and variance, skewness of trait distributions can indicate the existence of selective forces that structure ecological communities (Gaedke & Klauschies, 2017). Thus, D'Agostino skewness test (D'Agostino, 1970) was used to estimate the skewness of the trait distributions (package "moments" (Komsta & Novomestky, 2015)). All analyses were performed in R 3.4.0 (R Core Team 2017).

Table 1. Loadings based upon covariance matrix of first two principal components (PC1-PC2).

Measurement	PC1 (spine length)	PC2 (body size)
Dorsal spine 5	0.03	0.06
Dorsal spine 6	0.03	0.07
Dorsal spine 7	0.03	0.07
Dorsal spine 8	0.02	0.05
Lateral spine 8	0.03	0.05
Lateral spine 9	0.04	0.07
Pro-femur	0.11	0.01
Pro-tibia	0.14	0.01
Meso-femur	0.16	-0.01
Meso-tibia	0.18	-0.01
Meta-femur	0.2	-0.03
Meta-tibia	0.28	-0.02

4. 4 Results

4.4.1 Trait mean differences between fish-lake and dragonfly-lake populations

Populations from fish lakes had longer dorsal and lateral abdominal spines than populations from dragonfly lakes (PC spines: χ^2=20.90, df= 1, p<0.001) (Fig. 2A, Fig. 3A). Body size was larger in fish-lake populations than in dragonfly-lake population (PC body size: χ^2=6.24, df= 1, p=0.012) (Fig. 2B, Fig. 3B).

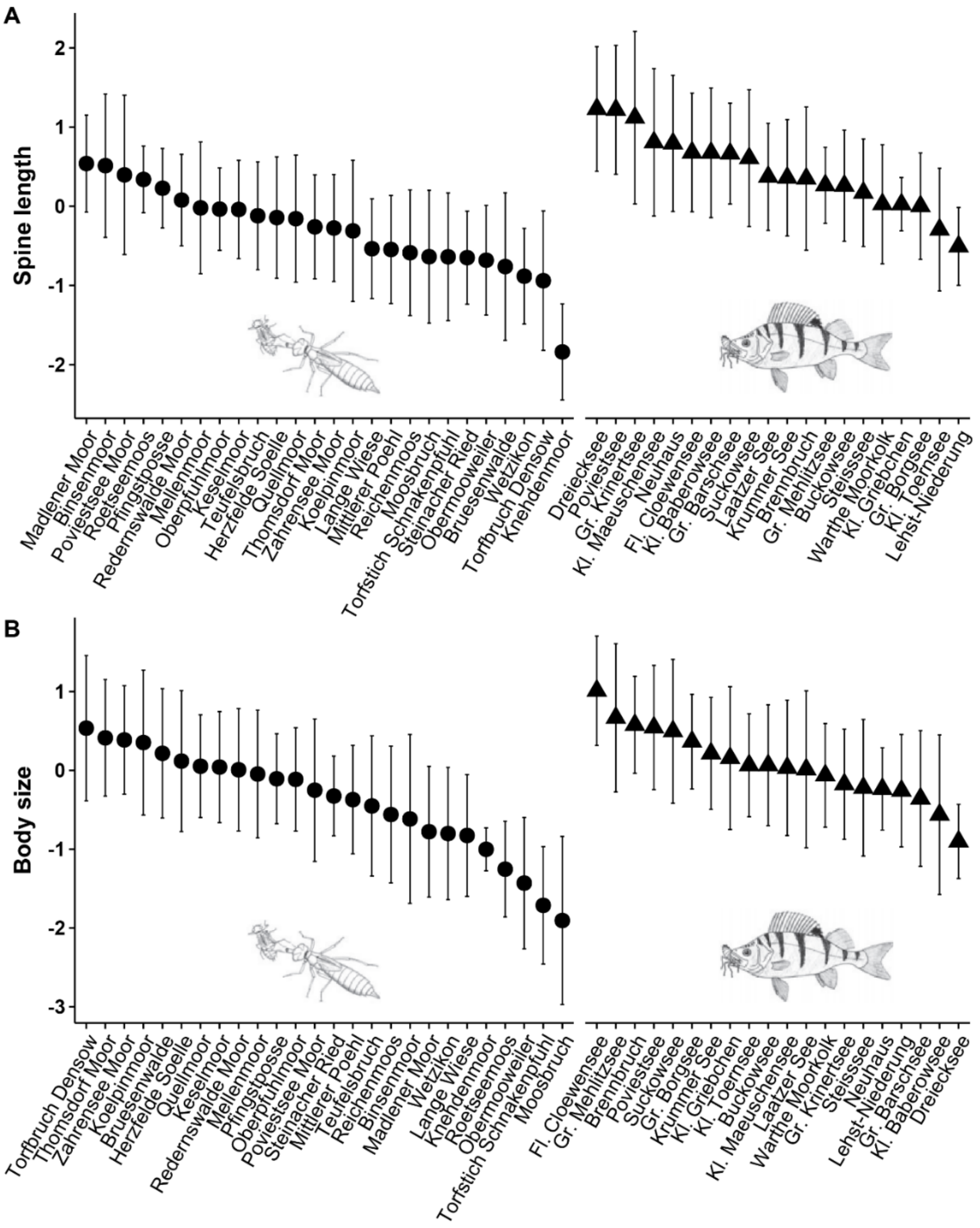


Fig. 2 Means ± SD of (A) spine length (PC spines) and (B) body size (PC body size) of each sampled dragonfly-lake (circle) and fish-lake (triangle) population. Fish and dragonfly larvae symbols also indicate different predation regimes for populations.

4.4.2 Trait variance differences between fish-lake and dragonfly-lake populations

1) <u>Total variance</u>: Total variance for spine length was significant larger in fish-lake populations than in dragonfly-lake populations (PC spines: $F_{1,1619}$=4.00, p=0.046; Fig 4A).

Total variance for body size was significantly larger in dragonfly-lake populations than in fish-lake populations (PC body size: $F_{1, 1619}$=11.12, p=0.024; Fig. 4B).

2) Between-population variance: Between-population variance on spine length (PC spines: $F_{1,44}$=0.10, p=0.757) and body size (PC body size: $F_{1,44}$=3.40, p=0.072) did not differ between fish-lake and dragonfly-lake populations (Fig. 4A and 4B).

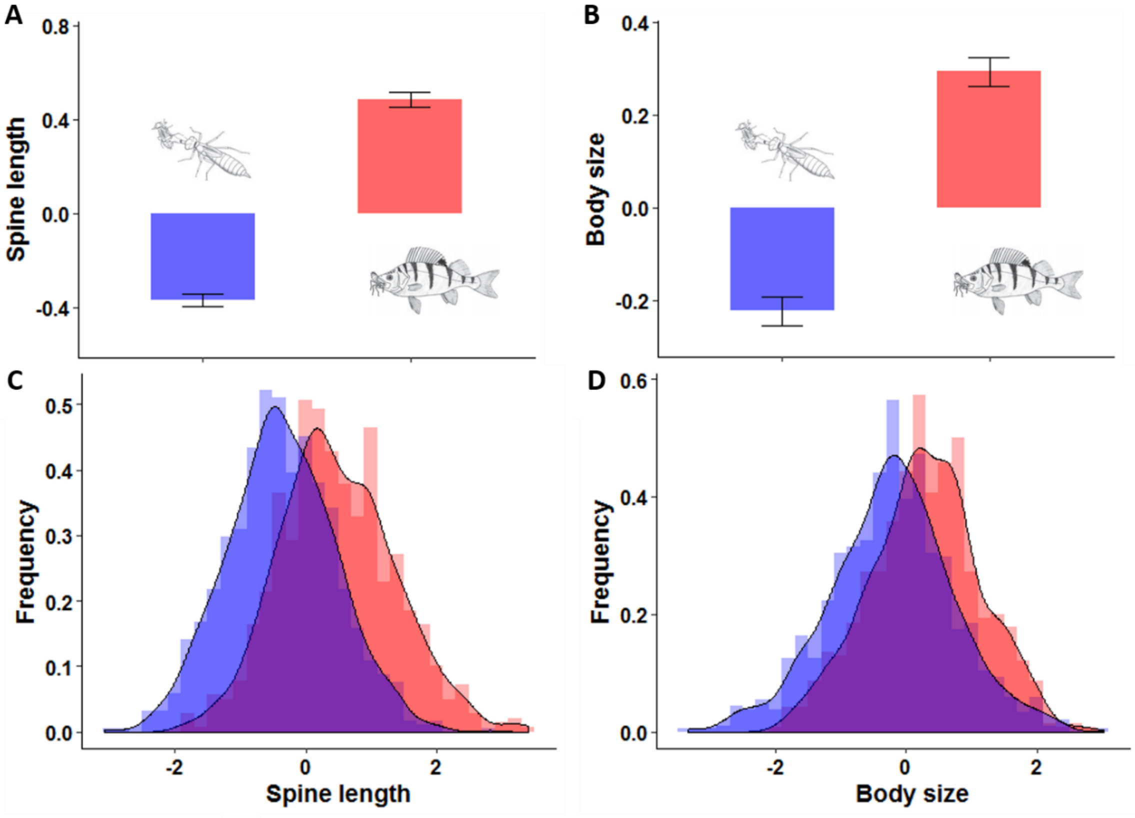


Fig. 3 Mean (±1SE) for (A) spine length (PC spines) and (B) body size (PC body size) as well as frequency distribution of (C) spine length (PC spines) and (D) body size (PC body size) for fish lakes indicated in red and dragonfly lakes indicated in blue. Fish and dragonfly larvae symbols indicate different predation regimes for populations.

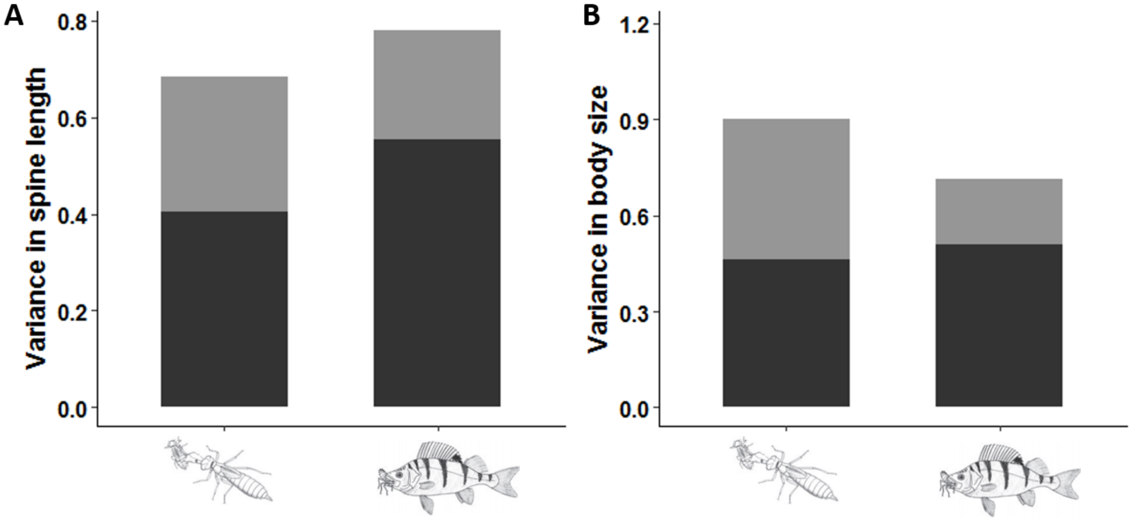


Fig. 4 Variance decompositions of (A) spine length (PC spines) and (B) body size (PC body size) between dragonfly lakes and fish lakes (indicated by drawing). Light grey indicates between-population variance and black indicates within-population variance.

4.4.3 Traits distribution visualization

By comparing trait distributions, we found that trait distributions of PC spine length did not show skewness (skewness=0.14, p=0.13; Fig 3C) in fish-lake populations. However, in dragonfly-lake populations, trait distributions of PC spine length showed a positive skewness (skewness=0.22, p=0.01; Fig 3C). Distribution of PC body size did not show skewness in both fish-lake populations (skewness= -0.10, p=0.26; Fig 3D) and dragonfly-lake populations (skewness= -0.03, p=0.70; Fig 3D).

4.5 Discussion

Predation can change the mean and variance of anti-predator traits among different populations (Poléo et al., 1995; Lahti et al., 2009; Runemark et al., 2014). Both mean and variance are crucial to understand community ecology (Violle et al., 2012). In our study, by analyzing large data set of *L. pectoralis* exuviates, we found that *L. pectoralis* from dragonfly lakes evolved shorter spines and smaller body size than individuals from fish lakes. Moreover, we also found that dragonfly-lake population reduced variance of spines trait contrasting to fish-lake population, but had larger variance of body size than fish-lake population. This suggest that change of predation regimes has big influences on the distribution of prey traits.

Long spines provide protection against fish for *Leucorrhinia* larvae (Johansson & Mikolajewski, 2008). In *Leucorrhinia* species, *L. dubia*, *L. glacialis*, *L. rubicunda*, and *L. intacta* were found having longer spines when they live with fish predators (Johansson & Samuelsson, 1994; McCauley et al., 2008; Petrin et al., 2010). Here, we show that *L. pectoralis* also has longer spines in fish lakes than in non-fish lakes. Additionally, *Leucorrhinia* species (e.g. *L. caudalis* and *L. albifrons*) with long dorsal and lateral spines always survive well in fish lakes, while species with short or no spines (e.g. *L. rubicunda* and *L. dubia*) always prefer non-fish lakes (Hovmöller & Johansson, 2004). Thus, in *Leucorrhinia*, intraspecific spine trait divergence mirrors patterns of phenotypic divergence among species, which means that spines trait played an essential role in *Leucorrhinia* speciation events (Schlichting & Wund, 2014). Unfortunately, we are not sure whether spine trait divergence is caused by phenotypic plasticity or genetic changes. Experiments for *L. dubia* (Johansson, 2002) and *L. intacta* (McCauley et al., 2008) proved that the spine length different comes from fish-induced phenotypic plasticity. For *L. pectoralis*, common garden experiments are also needed to solve this problem. However, because *L. pectoralis* adults are strong fliers (Corbet, 1999), it is very likely that there are gene flows among populations. This kind of genetic structure has be proved in *L. dubia* populations all over Europe continent except for population from Swiss Alps (Johansson et al., 2017). Thus, genetic differentiation might less likely happen among *L. pectoralis* populations in the same area; phenotypic plasticity must play an important role in this phenotypic diversification of *L. pectoralis*.

Change of predation pressure also influences traits variance within populations (Lahti et al., 2009; Runemark et al., 2014). Here, we found that spines had higher variance in fish-lake population than in dragonfly-lake population. Spines are effective traits against predatory fish, however, are selected against by large invertebrate predators (Mikolajewski et al., 2006). Reducing of variance in dragonfly-lake population may indicate a stabilization selection. Additionally, we also detected skewness of spine length distribution in dragonfly-lake population, which might suggest a sustained directional selection from invertebrate predations on spines (Jones et al., 2012). Moreover, skewness of the spine length distribution might also indicate a skewed fitness in dragonfly-lake populations (Urban et al., 2013). Therefore, when *L. pectoralis* shift the habitat from fish lakes to dragonfly lakes, a directional and stabilizing selection from new invertebrate predators drove the mean and variance change in spine traits. Higher variance in spine length of fish-lake population might suggest the heterogeneity of selection pressure from predatory fish (Pettorelli et al., 2015). In fish lakes, there are several different fish species (e.g. perch, crucian carp, pike, common roach, etc.) which can select for different length of spines. Moreover, aquatic macrophyte provides a safety microhabitat for aquatic invertebrates, which will indirectly change the risk of prey (Gilinsky, 1984; Thomaz & Cunha, 2010) (Henrikson, 1993).

Body size represents one of the most important quantitative traits under environmental selection (Blanckenhorn, 2000). Individuals with large body size can survive better with size-limited predation (Travis et al., 1985) and also can have a better foraging and escaping ability especially for dragonfly larvae as secondary predators in freshwater habitat. *L. pectoralis* populations from fish lakes grow larger body size than populations from dragonfly lakes. The possible reasons for these can be two sides. First, predatory fish (e.g. perch, crucian carp) are gape-limited predators (Wellborn et al., 1996) and prey body size affect predation efficiency of predators (Nilsson & Bronmark, 2000). Together with long spines, large body size will enable individuals to get more chances to escape from predatory fish. Although predation of large invertebrate predators are also size-limited, *Leucorrhinia* species cannot grow bigger than large invertebrate predators (e.g. *Anax*, *Ashna* larvae). Therefore, individuals with large body size might survive better in fish-lake populations. Second, predatory fish have a big impact on aquatic macro-arthropod communities (Wellborn & Robinson, 1991). Large invertebrate predators (e.g. *Anax*, *Ashna* larvae) are always eliminated in fish lakes because of the existence of predatory fish (McPeek, 1990b). To *L. pectoralis*, large invertebrate predators are not only predators but also competitors. Thus, with reduced large invertebrate predators in fish lakes, *L. pectoralis* might acquire more food resources. Similarly, improvement of body size was also found in crucial carp with predatory perch by reducing the intraspecific competition (Tonn et al., 1992).

Resource level have an effect on body size variation by changing growth rate (Jobling, 1983; Uchmański, 1985), which might be the reason that dragonfly-lake populations had larger variance of body size than fish-lake populations. As we discussed above, predatory fish might reduce the density of competitors for *L. pectoralis* and increased the resources level. Consequently, this can increase the mean of body size and decreased the variance of body size in fish-lake population in contrast to dragonfly-lake population. Increased variance via low food resources were also found in wood frog tadpoles (Wilbur & Collins, 1973; Peacor & Pfister, 2006). However, distribution of body size did not show a skewness like spines. This indicates that selection mode induced by top-predator shift is not identical between body size and spine traits (Bonamour et al., 2017).

Researches on phenotypic selection on quantitative traits in the wild are still urgently needed (Kingsolver & Diamond, 2011). The special life history of dragonflies, such as strong disperse ability in adults and isolation of larvae in lakes, could provide an opportunity to disentangle phenotypic selection mechanisms (Bybee et al., 2016). We here found that *L. pectoralis* from dragonfly-lake populations showed shorter spines and smaller body size than from fish-lake populations. The selection pressure from invertebrate predators also reduced the variance of spines in *L. pectoralis* in contrast to predatory fish. This results highlight intraspecific divergence can be as strong as interspecific divergence (interspecific divergence in spines showed in (Hovmöller & Johansson, 2004; Johansson & Mikolajewski, 2008)). Further, change of mean and variance in antipredator spines may indicate directional and stabilizing selection in *Leucorrhinia*-predator system (Lande & Arnold, 1983; Kingsolver et al., 2012).

4.6 Acknowledgements

I would like to thank Huidong Li for helping with Fig. 1 and J. Rolff for providing valuable comments on an earlier draft of this part. Axel Conrad did the drawings in Fig. 2-4.

4.7 Appendix

Table S1. Sampled populations of *Leucorrhinia pectoralis* (location) indicating sampling year, predation regime of the sampling lake (lake type), and number of exuviae per year (sample size).

Location	Sampling year	Lake type	Sample size
Wetzikon	2012/2013	Dragonfly lake	29/60
Binsenmoor	2008	Dragonfly lake	22
Brennbruch	2003/2005/2006	Fish lake	4/18/2
Bruesenwalde	2004/2013	Dragonfly lake	23/58
Buckowsee	2005	Fish lake	16
Dreiecksee	2013	Fish lake	3
Fl. Cloewensee	2012	Fish lake	84
Gartenweiher Neuhaus	2001	Fish lake	9
Gr. Borgsee	2006/2007/2008	Fish lake	2/7/39
Gr. Krinertsee	2011	Fish lake	11
Gr. Mehlitzsee	2008	Fish lake	4
Gr. Barschsee	2002/2004	Fish lake	10/3
Roetseemoos	2012	Dragonfly lake	19
Herzfelde Sölle	2009	Dragonfly lake	36
Kesselmoor	1996/1997	Dragonfly lake	57/81
Kl. Baberowsee	2002/2011	Fish lake	1/7
Kl. Griebchen	1998/2003	Fish lake	6/1
Kl. Maeuschensee	2013	Fish lake	50
Kl. Toernsee	2002	Fish lake	26
Suckowsee	2001	Fish lake	86
Knehdenmoor	2008	Dragonfly lake	6
Koelpinmoor	2011	Dragonfly lake	48
Krummer See	2009/2011/2012/2013/2015	Fish lake	13/33/8/27/15
Laatzer See	2003/2004	Fish lake	4/6
Lange Wiese	2009/2012	Dragonfly lake	5/14
Lehst-Niederung	2006	Fish lake	16
Madlener Moos	2012	Dragonfly lake	19

Mellenmoor	1998	Dragonfly lake	18
Mittlerer Pöhl	2001	Dragonfly lake	108
Poviestsee Moor	2008	Dragonfly lake	6
Zahrensee Moor	2009	Dragonfly lake	8
Redernswalde Moor	1990	Dragonfly lake	27
Warthe Moorkolk	2002/2003/2005/2006	Fish lake	34/7/16/9
Moosbruch	2004/2005	Dragonfly lake	3/5
Oberpfuhlmoor	2006/2012	Dragonfly lake	7/9
Pfingstposse	1998	Dragonfly lake	6
Poviestsee	2013	Fish lake	89
Quellmoor	2010	Dragonfly lake	34
Reichermoos	2012	Dragonfly lake	14
Obermooweiler	2012	Dragonfly lake	19
Steinacher Ried	2012	Dragonfly lake	20
Steißsee	2000/2007/2013	Fish lake	18/2/11
Teufelsbruch	2011	Dragonfly lake	47
Thomsdorf Moor	2012	Dragonfly lake	45
Torfbruch Densow	2013	Dragonfly lake	54
Torfstich Schnakenpfuhl	2010/2013	Dragonfly lake	5/14

Chapter V

Predator regime shift alters developmental trajectories of antipredator trait formation

5.1 Abstract

Predation is a major factor driving prey trait diversification and promoting ecological speciation. Antipredator traits are widely studied among prey species. However, it is unclear how different predators shape the development of antipredator traits. Species of the dragonfly genus *Leucorrhinia* underwent well-studied shifts from habitats being dominated by predatory fish (fish lakes) to habitat being dominated by predatory invertebrates (dragonfly lakes). Spines in larval dragonfly are efficient traits against fish predators. In this study, we compared the development curves of defensive spines in five species of *Leucorrhinia* dragonfly larvae. The time when each species reduce the spine growth rate was calculated. We found that after shifting into dragonfly lakes, *Leucorrhinia* species decreased the spine growth rate earlier than fish-lake species. For fish-lake species, spines and body size might evolved concomitantly to increase the body dimension against gape-limited fish predators. Our results highlight that developmental control of time points to initiate, slow down and complete inducible traits are essential to understand antipredator morphologies.

Key words: predator–prey interactions; antipredator traits; ontogenetic pattern; spines; developmental control.

5.2 Introduction

Predation is one of the major selective pressure driving species trait diversifications (Schluter, 2009). Antipredator traits diversification is widely studied in morphology, behavior, physiology and life history (Tollrian & Harvell, 1999; Lass & Spaak, 2003; Benard, 2004; Mitchell et al., 2017). However, the development of inducible defenses during preys' lifetime are largely overlooked (West-Eberhard, 2003; Hoverman & Relyea, 2007). Understanding time points of initiating and completing the inducible morphologies will permit a more complete understanding of antipredator strategies (Boege & Marquis, 2005; Hoverman & Relyea, 2007).

Phenotypic evolution of morphology occurs either by changes in sizes or proportions of body parts (Nijhout & German, 2012). Developmental energy allocation generates growth constrains among different body parts (Nijhout & Emlen, 1998). With limited energy inside body, allocation of energy to one part will diminishes the availability of energy to the other (Reznick, 1985). Moreover, because of heterogeneity of environmental selection pressures, relative growth of specific body parts can be reprogrammed during the development and hence, the growth trajectory of body parts will be changed (Wheeler, 1991; Moczek & Nijhout, 2002). New sets of nonlinear allometries, such as Logistic or Gompertz growth models, can be used to analyze the changes of the growth trajectory during the evolution of the phenotype (Nijhout & German, 2012; Paine et al., 2012). This might show us a clue how predation changes the growth trajectory of antipredator traits.

The spine of dragonfly larvae is prominent antipredator traits against fish predators (Mikolajewski & Johansson, 2004; Johansson & Mikolajewski, 2008). Possessing of long spines or not has a strong influence on the distribution of larvae stage among dragonfly species, especially in well-studied *Leucorrhinia* species (Odonata: Libellulidae) (Petrin et al., 2010). Long spines species prefer lakes with predatory fish as top predators (hereafter fish lakes), while short spines or no spine species prefer lakes without fish predators (but with large invertebrate predators, hereafter dragonfly lakes). Ancestrally occurred in fish lakes, *Leucorrhinia* species reduced the length of spines after inhabiting dragonfly lakes (Hovmöller & Johansson, 2004; Petrin et al., 2010). As fish are always gape-limited predators, long spines enlarge the body volume of dragonfly larvae and increase the handling time of fish predators (Price et al., 2015). Contrastively, invertebrate predators select against spines and larvae with long spines reduce the survival when facing invertebrate predators (Mikolajewski et al., 2006). Therefore, regulation of spines elongation should be different between species from fish lakes (fish-lake species) and species from dragonfly lakes (dragonfly-lake species). Although the distinctive evolution of spines length between fish-lake species and dragonfly-lake species is known (Hovmöller & Johansson, 2004), the evolution of developmental pattern of spines due to habitat shift have not been examined.

Here, we investigated developmental scaling relationship of spines length to body size in five European *Leucorrhinia* species. Specifically, we want to clarify whether *Leucorrhinia* species from different predator regime habitats evolved different developmental constraints on elongation of antipredator spines. We expected that dragonfly-lake species should evolved lower growth rate of spines or decrease spine growth rate earlier than that in fish-lake species.

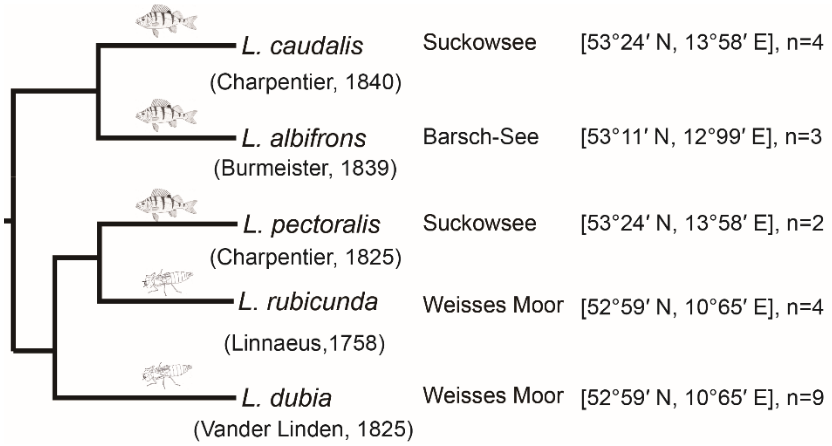


Fig. 1 Phylogeny of five European *Leucorrhinia* species modified from Hovmöller and Johansson (2004). Fish and dragonfly larvae symbols indicate the preferred top predator for each species. Sample locations, whether the lakes contain predatory fish or not, and the number of egg clutches collected are given for each species.

5.3 Material and methods

5.3.1 Colleting and housing

At least two egg clutches for each species were collected during June, 2016 (details in Fig. 1). Egg clutches were kept separately in a floating container until they hatched (usually take around 2 weeks). Hatched larvae from the same species were mixed and put into big buckets with aged-tape water (~9L). Larvae were raised with *ad libitum* amount of daphnia. All the buckets were kept outside with natural temperature and light regimes (Berlin, Germany: [52 °31' N and 13 °24' E]; Fig. 2).

Fig. 2 The buckets in the garden (Freie Universität berlin, Zoologie department, June 2017): A) buckets for keeping egg clutches to hatch; B) buckets for keeping hatched larvae.

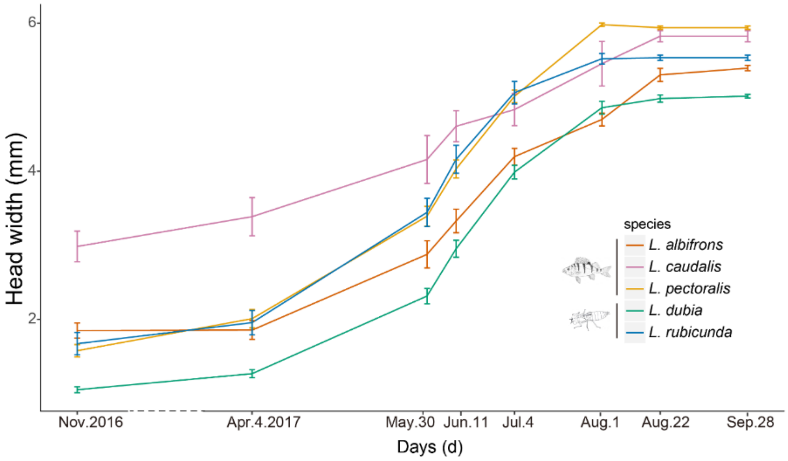


Fig. 3 Examine time points and growth line of each species. Presented are mean ±1.95 SE. Species and the top predator in their preferred habitat are also indicated.

5.3.2 Inspection and measurements

Larvae are inspected at several time points (Fig. 3). For each time, we counted the number of larvae survived and took photos of each larva. Larvae were reallocated within each species to make the density in each bucket to be equal (around 10-15 larvae per bucket from April, 2017 on). The instar of the larvae was also examined. For each larvae, we measured head width and lateral spines in segment 8 and 9. Head width was used as a proxy of body size (Benke, 1970).

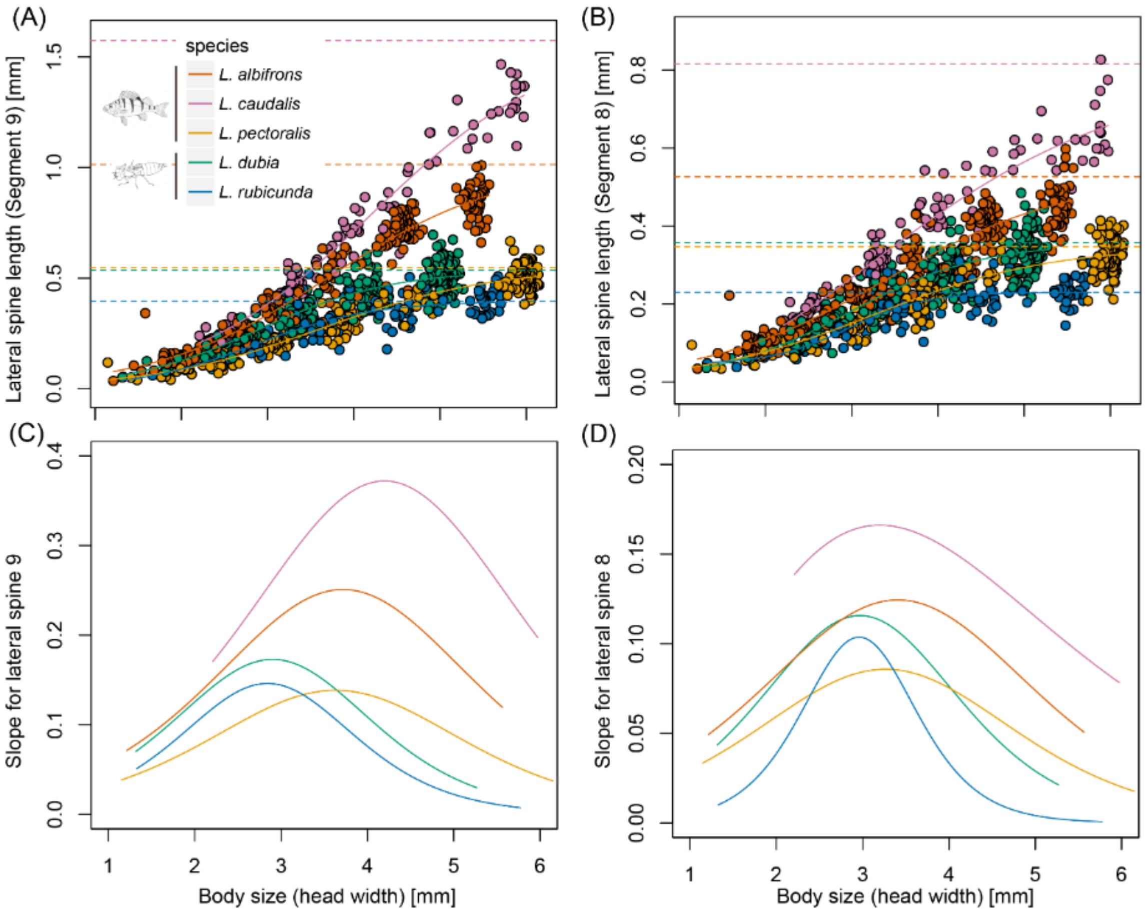


Fig. 4 Scaling relationships of lateral spines length and Body size during larval developing time.

5.3.3 Data analysis

First, difference in head width of last instar larvae were examined by one-way ANOVA with Tukey’s test for multiple comparisons.

Secondly, we examined the scaling relationship between lateral spines (spines in segment 8 and 9 separately) and head width with different growth models. Models include linear model and non-linear models (exponential, power law, monomolecular, logistic and Gompertz model). Detailed model descriptions are showed in supplementary material Table S1 (Paine et al., 2012). Most suitable models were selected according to AIC values.

The head width value with highest slope in the regression (hereafter transition size) were extracted from the best models. Transition size was selected because from this point on spine growth rate slowed down in relative to the enlargement of body.

We tested whether habitat shift from fish lakes to dragonfly lakes caused an evolutionary change on transition size of antipredator spines. Comparative analyses were carried out with Phylogenetic Generalized Least Squares (PGLS) in "caper" package (Orme, 2013). In PGLS, species number represent the sample size, with preferred habitat (fish lake versus dragonfly lake) being considered as the independent variable, and transition size of lateral spines in segment 8 and 9 as the dependent variable. We incorporated branch length from the pruned *Leucorrhinia* phylogeny (Hovmöller pers. Communication, Fig. 1) (Hovmöller & Johansson, 2004).

5.4 Results

Among all five *Leucorrhinia* species, last instar larvae differed in head width among species (one-way ANOVA: $F_{4,657}$=1949, $P<0.001$). Species differed from each other significantly (Tukey's test: all $P<0.001$), with head width from high to low: *L. pectoralis*, *L. caudalis*, *L. rubicunda*, *L. albifrons*, and *L. dubia* (Fig. 3, Supplementary materials Fig. S1).

According to AIC values, three-parameter logistic model was the most suitable model for scaling relationship of spines in segment 9 across all species (Supplementary material Table S2). For spines in segment 8, four-parameter logistic model was the most suitable model in *L. caudalis*, Gompertz model in *L. rubicunda*, while the other species were all fit with three-parameter logistic model (Supplementary material Table S2).

Comparative analyses with PGLS showed that both lateral spines in segment 9 and 8, the transition size in fish-lake species were significantly larger than dragonfly-lake species (spine in segment 9: $F_{1,3}$=23.19, P=0.02, λ=0; spine in segment 8: $F_{1,3}$=278.63, $P<0.001$, λ=0. Fig. 4). According to the size range of each instar, fish-lake species have the transition size at F-2 instar, while dragonfly-lake species at F-3 instar (Table1, Appendix Fig. S2).

Table 1 The corresponding instar when *Lecuorrhinia* species having largest spine growth rate.

Species	Top-predator	Head width with largest spine growth rate (mm)		Corresponding instar
		Lateral spine in segment 8	Lateral spine in segment 9	
L. albifrons	Fish	3.41	3.72	F-2
L. caudalis	Fish	3.50	4.20	F-3~F-2
L. pectoralis	Fish	3.43	3.63	F-3~F-2
L. dubia	Invertebrate	2.97	2.90	F-3
L. rubicunda	Invertebrate	2.96	2.84	F-3

5.5 Discussion

Our study demonstrates for the first time that predator change by habitat shift caused differences of scaling relationship between antipredator spines and body size in an ontogenetic view. Transition size of spines was decreased after *Leucorrhinia* species inhabiting dragonfly lakes, which means in order to generate short spines, dragonfly-lake species slowed down the development of spines in earlier instar than fish-lake species.

Dragonfly-lake species reduced the length of spines because invertebrate predators select against spines (Mikolajewski et al., 2006). To reduce the length of spines, species can either hold a low growth rate of spines or cease the development of spines early in their life stages. According to our results, dragonfly-lake species maintain low spine growth rate all through their larval stage. Additionally, dragonfly-lake species also terminate the increasing developing speed in their early instars. By reallocating of resources for development, this will save energy for the grow of other body parts (Nijhout & Emlen, 1998). Change of transition size in antipredator spines may indicate a change of developmental constrains among species groups from different predation regimes.

Predatory fish are mostly gape-limited predators (Hambright, 1991). Prey evolved large body dimensions can always survive better (Moodie, 1972). Existence of spines can help to enlarge the body dimensions and increase predator handling time (Nilsson & Brönmark, 2000; Price et al., 2015). Experiments in fish showed that spines and body size increase concomitantly in the presence of fish predators (Januszkiewicz & Robinson, 2007). This pattern is also showed in fish-lake species *L. caudalis*, which has both long spines and large body size. While we also found a compensatory relationship between spine length and body size in *L. pectoralis* (fish-lake species). This species owns short spines but the largest final body size among *Leucorrhinia* species. Therefore, even under the same predatory fish selection, fish-lake species can acquire different ways to achieve refuge size.

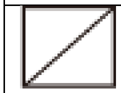
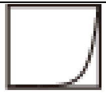
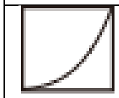
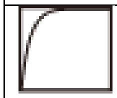
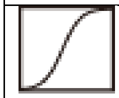
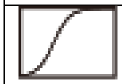
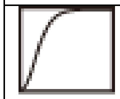
Overall, our results showed that predator change via habitat shift drives different developmental trajectories on antipredator traits. Thus, the influences from predators are not only can be seen on the final states but also on the developmental pattern of antipredator traits.

5.6 Acknowledgments

I like to thank G. Yu and J. Wu for providing comments on an earlier version of this part.

5.7 Appendix

Table S1 Models used for fitting scaling relationship between spines (S) and body size (H for head width). Absolute growth rate (AGR) is the derivative of S with respect to H, i.e. *d*S/*d*H. Relative growth rate (RGR) can be expressed either as a function of body size (H), i.e. (*d*S/*d*H)/H. Inflection point is the point of body size at which AGR is maximized.

Shape	Name	Description
	Linear	Constant AGR
	Exponential	Constant RGR
	Power law	RGR will slow down with increasing of body size
	Monomolecular	AGR is fast initially and slows thereafter
	Three-parameter logistic	Asymptotic regression; lower horizontal asymptote is fixed at 0, the inflection point falls at K/2 (K is asymptotic spine length)
	Four-parameter logistic	Loose one or the other of strictures in three-parameter logistic model
	Gompertz	Inflection point occurs at around 37% of asymptotic spine length K

Reference: Paine et al. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution*, 2012, 3, 245–256.

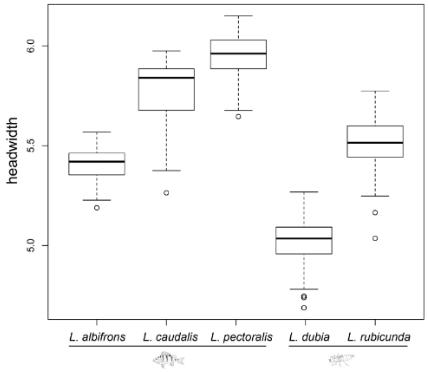


Fig. S1 Head width of last instar larvae comparisons among *Leucorrhinia* species.

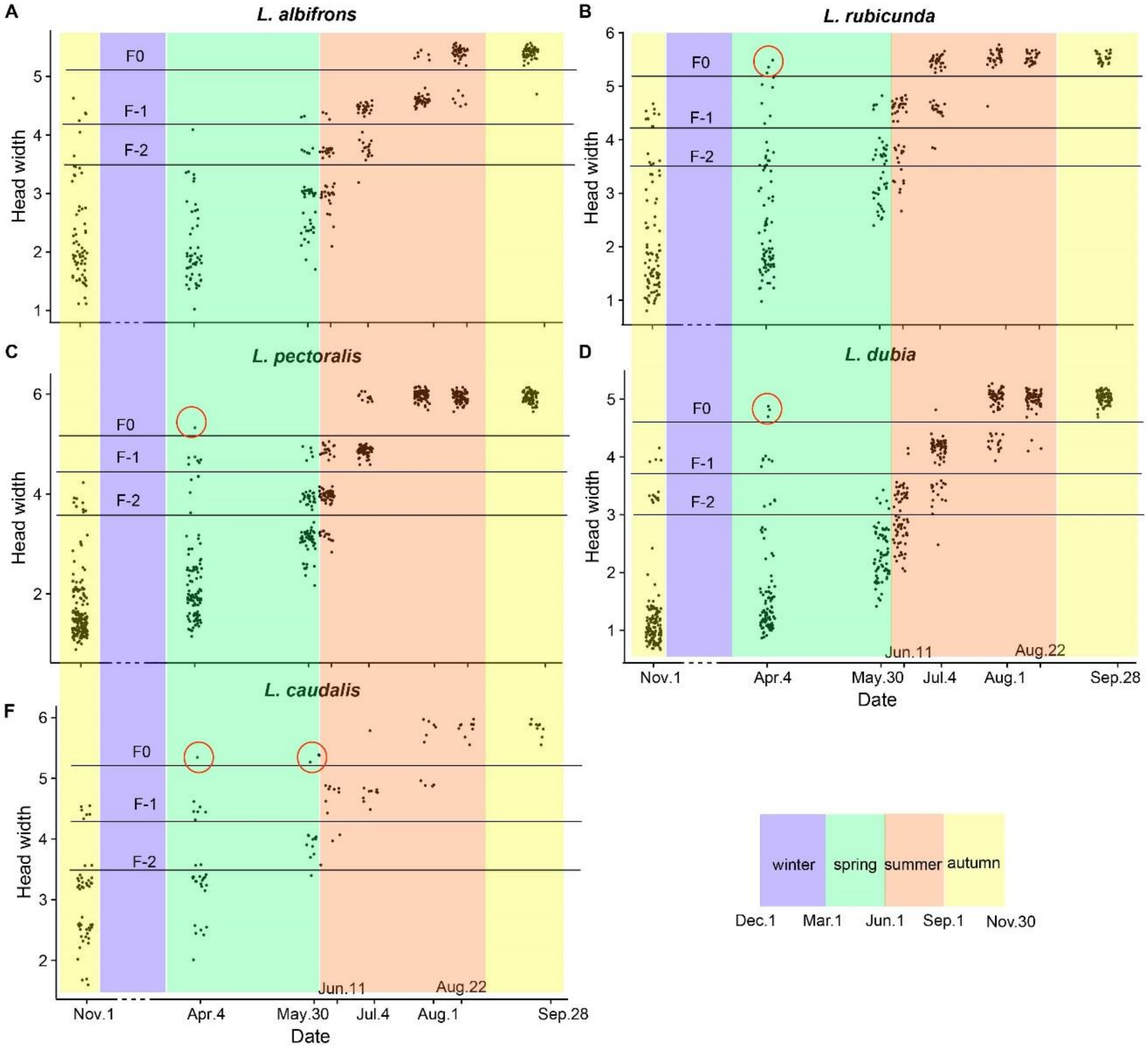


Fig. S2: Growth and instar distribution along different examining time. Season we used are Northern Meteorological seasons. Date indicated on x axis are the examining time points. Approximate instar is also indicated in the figure according to head width. Last instar larvae after the first winter are indicated with red circles. Each dot represents one larva at that time point and larvae are repeatedly examined during their whole life history.

Summary

Predator-prey interactions have a major influence on species diversification (Barbosa & Castellanos, 2005). The performance and fitness of prey species are heavily dependent on their antipredator responses to specific predators (Tollrian & Harvell, 1999). In nature, predators are distributed heterogeneously across different habitats (Wellborn et al., 1996). Because different predators vary in their predation strategies, a change in the top predators can dramatically alter preys' defensive traits. Larval *Leucorrhinia* ancestrally came from lakes dominate with predatory fish (fish lakes) (Hovmöller & Johansson, 2004). However, they shifted their habitats from fish lakes into lakes with only large invertebrate predators (dragonfly lakes) several times. In Europe, two of the five *Leucorrhinia* species shifted into dragonfly lakes and evolved a distinctive pattern of antipredator traits. In this thesis, I examined a series of antipredator traits in order to understand how different predation regimes drive prey trait diversification in European *Leucorrhinia* species.

Pre- and post-contact antipredator traits

Leucorrhinia larvae adopt both pre- and post-contact antipredator traits. According to results from chapters I and II, antipredator behavior and morphology are widespread in all five *Leucorrhinia* larvae. Pre-contact behaviors such as walking, swimming and foraging were increased in species from dragonfly lakes (dragonfly-lake species) in contrast to species from fish lakes (fish-lake species). A low level of foraging and moving behavior in fish-lake species reduces the chance of getting detected by high speed predatory fish (McPeek, 1990a), however, the reduction in behavior is associated with the cost of low food acquisition in nature. Moreover, antipredator behaviors in *Leucorrhinia* from two predation regimes tend to only react to native top-predators in their habitat, i.e. dragonfly-lake species responding to predatory invertebrate only but not to fish predators. This indicates that dragonfly-lake species lose the ancestral ability to regard fish as a predation threat.

Once *Leucorrhinia* larvae have been detected and attacked by a predator, post-contact traits, such as spines and a fast escape swimming speed, give the larvae an increased chance of surviving (Mikolajewski & Johansson, 2004). A top-predator related habitat shift results in a change of the selection pressure, which consequently induces changes in a suite of antipredator traits (behavior and escape traits in chapter II). In contrast to fish-lake species, dragonfly-lake species have a low burst escape speed, which is correlated with having a narrow abdomen, reduced muscle mass and small branchial chamber size. These results suggest that antipredator traits are strongly correlated with each other. Further, after predator regime shift, there may be relaxed selection on those changed antipredator traits.

Growth rate: what really matters

Growth rate affects many fitness-related traits, such as body size, maturation age, reproductive ability, stress tolerance and longevity (McCay et al., 1935; Gotthard et al., 1994; Walsh & Reznick, 2010). Thus, growth rate is under strong natural selection. Changes in growth rate in response to predation are due to at least two main factors: foraging behavior and digestive traits (e.g. food assimilation efficiency, growth efficiency). In chapter III, I found that growth rate did not follow the foraging behavior pattern among five European *Leucorrhinia* species (in chapter I: dragonfly-lake species increased foraging behavior, which would be assumed to result in higher food accessibility and enhanced growth rate). Both fish-lake and dragonfly-lake species showed similar growth rate, but they differed in their digestive traits. For fish-lake species, digestive traits might compensate for the negative effects of low foraging behavior (Stoks et al., 2012). However, *L. dubia* showed a distinct digestive traits from fish-lake species rather than *L. rubicunda*. This phenomenon might be explained by unique evolutionary history and the abiotic tolerance of *L. dubia*. All in all, no matter what the main factor is that controls growth rate, *Leucorrhinia* species can achieve similar growth rates in diversified ways. Additionally, *Leucorrhinia* species tend to increase their growth rate in the presence of predators, which might reflect the existence of a size selection pressure or developmental time pressure from both predators.

Mean and variance: predation shapes community ecology

Predation changes the mean and variance of prey traits (Runemark et al., 2014; Nakagawa et al., 2015), which both have large ecological effects (e.g. niche breadth, species interaction) (Bolnick et al., 2011). Long defensive spines improved the survival of larvae inhabiting fish lakes (Mikolajewski & Johansson, 2004). Contrasting spine trait divergence exists among *L. pectoralis* populations (Chapter IV) as well as among all *Leucorrhinia* species (Johansson & Mikolajewski, 2008) from different predation regimes. Body size is frequently used as a surrogate for fitness and larvae with large body size are supposed to have higher fitness (Blanckenhorn, 2000; Dmitriew, 2011). Populations of *L. pectoralis* from fish lakes also have larger body size than populations from dragonfly lakes. This advantage for the larvae in fish lakes might transfer into a large advantage for adult fitness, such as fecundity (Honěk, 1993). Moreover, larval *L. pectoralis* from fish lakes had larger variance in spine length than populations from dragonfly lakes but had lower variance in body size than dragonfly-lake populations. This indicates that different traits have different level of response to predation. Reduced spine variance in dragonfly-lake populations may indicate the possibility of stabilizing selection from invertebrate predators. Existence of predatory fish might reduce the competitors of *L. pectoralis* and increased food resources, which could be the reason for increasing mean value and decreasing variance of body size in fish-lake populations simultaneously.

Developmental trajectories of antipredator spine formation

The importance of development in the formation of inducible defenses is largely overlooked (West-Eberhard, 2003; Hoverman & Relyea, 2007). Predation can not only drive phenotypic divergence in the final state of antipredator traits but also have an impact on the development of antipredator traits. In *Leucorrhinia*, predatory fish and invertebrate predators have antagonistic selection effects on spines (Mikolajewski et al., 2006). Due to this, fish- and dragonfly-lake species showed different developmental trajectories of antipredator spine formation. In chapter V, I measured the development of defensive spine length along the increase of body size. Results suggested that after *Lecuorrhinia* species shift into dragonfly lakes, they decreased spine growth rate one instar earlier (F-3 instar) than fish-lake species (F-2 instar). Fish-lake species do not all keep high spine growth rate (especially in *L. pectoralis*). However, larval *L. pectoralis* have the largest body size. Therefore, both long spines and large body size might contribute to enlarge larval body dimension against gape-limited predatory fish. All in all, developmental approaches provide a more complete understanding of induced antipredator traits.

Outlook and further perspectives

In this thesis, I used European *Leucorrhinia* species as a model to study predator-induced prey diversification. Although mechanisms behind phenotypic divergence are unknown, these research I have presented is critical for improving our understanding of adaptive trait plasticity and its widespread occurrence across species and community types (Kishida et al., 2009). Moreover, the facts that the larval stage is restricted in its distribution and that the adults have a high dispersal ability make odonates as an important model bridging ecology and evolution (Bybee et al., 2016). My results indicate that it might be possible to disentangle the forces behind natural selection (e.g. directional selection, stabilizing selection) by using *Leucorrhinia* species.

Zusammenfassung

Räuber-Beute-Wechselwirkungen haben einen großen Einfluss auf die Artenvielfalt (Barbosa & Castellanos, 2005). Die Leistung und Fitness von Beutetieren ist stark abhängig von ihren Antipredatorstrategien gegen spezifische Räuber (Tollrian & Harvell, 1999). In der Natur sind Räuber heterogen über verschiedene Lebensräume verteilt (Wellborn et al., 1996). Da sich verschiedene Prädatoren in ihren Räuberstrategien unterscheiden, kann eine Änderung der Top-Prädatoren die Verteidigungsstrategien der Beute dramatisch verändern. Larven der Gattung *Leucorrhinia* stammen ursprünglich aus Seen, die von Raubfischen (Fischseen) dominiert sind (Hovmöller & Johansson, 2004). Im Laufe der Zeit verschob sich ihr Lebensraum mehrmals von Fischseen zu Seen mit nur großen wirbellosen Raubtieren (Libellenseen). Zwei der fünf *Leucorhininia*-Arten haben ihr Habitat in Europa in Libellenseen verschoben und evolvierten ein distinktes Muster von Antipredator-Eigenschaften aus. In der vorliegenden Arbeit untersuchte ich eine Reihe von Antipredatorstrategien, um zu verstehen, wie unterschiedliche Prädationsregimes die Divergenz bei europäischen *Leucorrhinia*-Arten lenken.

Prä- und Postkontakt-Antipredator-Strategien

Leucorrhinia-Larven zeigen sowohl Prä- als auch Post-Kontakt-Antipredator-Strategien. Nach den Ergebnissen meines ersten und zweiten Kapitels ist in den Larven aller fünf *Leucorrhinia*-Arten anti-predatorisches Verhalten und anti-predatorische Morphologie weit verbreitet. Prä-Kontaktverhalten wie Gehen, Schwimmen und Nahrungssuche waren bei Arten von Libellenseen (Libellenseen) im Gegensatz zu Arten von Fischseen (Fischseesorten) stärker ausgeprägt. Durch ein vermindertes Nahrungs- und Bewegungsverhalten in Fischsee-Arten ist es weniger wahrscheinlich, durch schnell jagende Raubfische entdeckt zu werden (McPeek, 1990a), jedoch ist dies mit einer geringen Nahrungsaufnahme in der Natur verbunden. Außerdem reagieren *Leucorrhinia*-Arten aus zwei Prädationsregimen nur auf einheimische Top-Räuber in ihrem Habitat, d.h. es gibt Libellensee-Arten, die nur auf wirbellose Räuber reagieren, aber nicht auf

Fischprädatoren. Dies gibt einen Hinweis darauf, dass Libellenarten ihre ursprüngliche Fähigkeit verloren, Fische als Bedrohung durch Raubtiere zu betrachten.

Sobald *Leucorrhinia*-Larven von einem Räuber entdeckt und angegriffen wurden, haben die Larven eine erhöhte Überlebenschance durch Post-Kontakt-Eigenschaften, wie Stacheln und eine schnelle Fluchtgeschwindigkeit (Mikolajewski & Johansson, 2004). Eine Habitatverschiebung durch Top-Predatoren führt zu einer Veränderung des Selektionsdrucks, der folglich zu Veränderungen in einer Reihe von Antipredatorstrategien führt (Verhaltens- und Fluchtmerkmale in Kapitel II). Im Gegensatz zu Fischsee-Arten haben Libellensee-Arten eine geringere Fluchtgeschwindigkeit, die mit einem schmalen Abdomen, reduzierter Muskelmasse und kleiner Branchialkammergröße korreliert. Außerdem kann es nach einer Verschiebung des Räuberregimes zu einem geringeren Selektionsdrucks auf diese veränderten Antipredatorstrategien kommen.

Wachstumsrate: Was wirklich zählt

Die Wachstumsrate beeinflusst viele Fitnessmerkmale wie Körpergröße, Reifungsalter, Fortpflanzungsfähigkeit, Stresstoleranz und Lebensdauer (McCay et al., 1935; Gotthard et al., 1994; Walsh & Reznick, 2010). Daher steht die Wachstumsrate unter starker natürlicher Selektion. Veränderungen in der Wachstumsrate als Reaktion auf Prädation sind auf mindestens zwei Hauptfaktoren zurückzuführen: Nahrungssuche und Verdauung (z. B. Nahrungsmittelassimilationseffizienz, Wachstumseffizienz). In Kapitel III stellte ich fest, dass die Wachstumsrate nicht dem Muster der Nahrungssuche der fünf europäischen *Leucorrhinia*-Arten folgte (in Kapitel I: Libellensee-Arten erhöhen die Nahrungssuche, was zu höherer Nahrungsverfügbarkeit und einer verbesserten Wachstumsrate führen kann). Sowohl Fischsee- als auch Libellensee-Arten zeigten ähnliche Wachstumsraten, unterschieden sich jedoch in ihren Verdauungsmerkmalen. Bei Fischsee-Arten könnten verdauungsfördernde Eigenschaften die negativen Auswirkungen eines geringen Nahrungssuchverhaltens kompensieren (Stoks et al., 2012). Jedoch zeigte *L. dubia* eine distinkte Verdauung von Fischsee-Arten anders als *L. rubicunda*. Dieses Phänomen könnte durch die einzigartige Entwicklungsgeschichte und die abiotische Toleranz von *L. dubia* erklärt werden. Alles in allem können *Leucorrhinia*-Arten, unabhängig von was der kontrollierende Hauptfaktor für die Wachstumsrate ist, ähnliche Wachstumsraten in unterschiedlicher Weise erreichen. Darüber hinaus tendieren *Leucorrhinia*-Arten dazu, ihre Wachstumsrate in Gegenwart von Räubern zu erhöhen, was die Existenz eines Größenselektionsdrucks oder Entwicklungszeitdrucks durch beide Räuber zeigt.

Mittelwert und Varianz: Predation prägt die Gemeinschaftsökologie

Prädation verändert den Mittelwert und die Varianz der Beutetiermerkmale (Runemark et al., 2014; Nakagawa et al., 2015). Beide haben große ökologische Auswirkungen(z. B. Nischenbreite, Arteninteraktion) (Bolnick et al., 2011). Lange Abwehrstacheln verbesserten das Überleben von Larven in Fischseen (Mikolajewski & Johansson, 2004). Gegensätzliche Divergenz der Abwehrsatcheln existieren innerhalb der *L. pectoralis*-Populationen (Kapitel IV) sowie innerhalb aller *Leucorrhinia*-Arten (Johansson & Mikolajewski, 2008) aus unterschiedlichen Prädationsregimen.

Als Ersatz für Fitness wird oft Körpergröße verwendet. Larven mit einer großen Körpergröße weisen eine höhere Fitness auf (Blanckenhorn, 2000; Dmitriew, 2011). Populationen von *L. pectoralis* aus Fischseen haben eine größere Körpergröße als Populationen von Libellenseen. Dieser Vorteil für die Larven aus Fischseen könnte sich als großer Vorteil für die Fitness der adulten Tiere, wie zum Beispiel erhöhter Fruchtbarkeit(Honěk, 1993), auswirken. Zudem zeigten *L. pectoralis* Larven aus Fischseen eine größere Varianz in der Länge ihrer Abwehrstacheln als Populationen aus Libellenseen. *L. pectoralis* Larven aus Fischseen zeigten jedoch eine geringere Varianz in ihrer Körpergröße als Populationen aus Libellen-Seen. Dies deutet darauf hin, dass verschiedene Merkmale unterschiedliche Reaktionen auf Prädation zeigen. Eine kleinere Varianz in den Abwehrstacheln in Populationen aus Libellen-Seen könnte darauf hindeuten, die Selektion von Wirbellosen-Räubern zu stabilisieren. Das Vorkommen von Raubfischen könnte zu geringerer Konkurrenz und erhöhtem Angebot an Nahrungsressourcen für *L. pectoralis* führen, was der Grund für die gleichzeitige Zunahme des Mittelwerts und der abnehmenden Varianz der Körpergröße in Fischseepopulationen sein könnte.

Entwicklungskurven der Bildung von Abwehrstacheln

Die Bedeutung der Entwicklung von induzierbaren Abwehrmechanismen bleibt häufig unbeachtet (West-Eberhard, 2003; Hoverman & Relyea, 2007). Prädation kann nicht nur die phänotypische Divergenz im Endstadium der anti-predatorischen Eigenschaften fördern, sondern auch die Entwicklung von anti-Räuber-Merkmalen beeinflussen. Räuberische Fische und wirbellose Raubtiere haben in *Leucorrhinia* antagonistische Selektionseffekte auf Abwehrstacheln (Mikolajewski et al., 2006). Aus diesem Grund zeigten die Arten der Fisch- und Libellenseen unterschiedliche Entwicklungsverläufe in der Entwicklung ihrer Abwehrstacheln. Im fünften Kapitel habe ich die Länge der Verteidigungsstacheln im Verlauf der Zunahme der Körpergröße gemessen. Meine Ergebnisse deuteten darauf hin, dass sich die Wachstumsrate der Abwehrstacheln innerhalb der *Lecuorrhinia*-Arten, nach ihrem Habitatswechsel zu Libellenseen, um ein Larvenstadium verlangsamt hat (F-3-Stadium) im Vergleich zu den Fischsee-Arten (F-2-Stadium). Fischsee-Arten zeigen nicht alle eine hohe Wachstumsrate ihrer Abwehrsatcheln (vor allem *L. pectoralis*). Die Larven von *L. pectoralis* haben jedoch die größte Körpergröße. Daher können sowohl lange Stacheln als auch große Körpergrößen dazu beitragen, größen-limitierten Raubfischen zu entkommen. Alles in allem bieten Studien zur Entwicklung ein vollständigeres Verständnis der induzierten Antipredatorstrategien.

Ausblicke

In der vorliegenden Arbeit habe ich europäische *Leucorrhinia*-Arten als Modellorganismen zur Untersuchung der räuberinduzierten Divergenz von Beutetieren verwendet. Obwohl die Mechanismen hinter der phänotypischen Divergenz unbekannt sind, sind die von mir vorgestellten Untersuchungen entscheidend für unser Verständnis von Plastizität adaptiver Eigenschaften und ihres weitverbreiteten Vorkommens innerhalb von Arten und Gemeinschaften (Kishida et al., 2009). Darüber hinaus machen die Fakten, dass Larven in ihrer Ausbreitung beschränkt sind und dass die adulten Tiere eine hohe Ausbreitungsfähigkeit haben, Libellen zu wichtige Modellorganismen, die Ökologie und Evolution verbinden (Bybee et al., 2016). Meine Ergebnisse zeigen, dass es möglich ist, natürlichen Selektion (z. B. direktionale Selektion, stabilisierende Selektion) durch die Verwendung von *Leucorrhinia*-Arten zu entwirren.

Bibliography

Abrams, P.A. (2000) Character shifts of prey species that share predators. *The American Naturalist*, **156**, S45-S61.

Abrams, P.A. & Matsuda, H. (1997) Prey adaptation as a cause of predator-prey cycles. *Evolution*, **51**, 1742-1750.

Abrams, P.A. & Rowe, L. (1996) The effects of predation on the age and size of maturity of prey. *Evolution*, **50**, 1052-1061.

Adams, D.C. & Otárola-Castillo, E. (2013) geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, **4**, 393-399.

Allen, D., Rosenfeld, J., & Richards, J. (2016) Physiological basis of metabolic trade-offs between growth and performance among different strains of rainbow trout. *Canadian journal of fisheries and aquatic sciences*, **73**, 1493-1506.

Anholt, B.R., Werner, E., & Skelly, D.K. (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology*, **81**, 3509-3521.

Anholt, B.R. & Werner, E.E. (1995) Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology*, **76**, 2230-2234.

Arendt, J. (2010) Morphological correlates of sprint swimming speed in five species of spadefoot toad tadpoles: comparison of morphometric methods. *Journal of Morphology*, **271**, 1044-1052.

Arnqvist, G. & Johansson, F. (1998) Ontogenetic reaction norms of predator-induced defensive morphology in dragonfly larvae. *Ecology*, **79**, 1847-1858.

Askew, R.R. (1988) The dragonflies of Europe. Harley books, Colchester, UK.

Barbosa, P. & Castellanos, I. (2005) Ecology of predator-prey interactions. Oxford University Press, Oxford, UK.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Grothendieck, G., Green, P., & Bolker, M.B. (2016) Package 'lme4'. Obtenido de http://pbil. univlyon1. fr/CRAN/web/packages/lme4/lme4. pdf.

Baxter-Gilbert, J., Riley, J.L., & Whiting, M.J. (2018) Runners and fighters: clutch effects and body size drive innate antipredator behaviour in hatchling lizards. *Behavioral Ecology and Sociobiology*, **72**, 97.

Beckerman, A.P., Wieski, K., & Baird, D.J. (2007) Behavioural versus physiological mediation of life history under predation risk. *Oecologia*, **152**, 335-343.

Benard, M.F. (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology Evolution and Systematics*, **35**, 651-673.

Benke, A.C. (1970) A method for comparing individual growth rates of aquatic insects with special reference to the Odonata. *Ecology*, **51**, 328-331.

Bestion, E., Clobert, J., Cote, J., & Gaillard, J.-M. (2015) Dispersal response to climate change: scaling down to intraspecific variation. *Ecology Letters*, **18**, 1226-1233.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, 385-407.

Blumstein, D.T. & Daniel, J.C. (2005) The loss of anti-predator behaviour following isolation on islands. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 1663-1668.

Blumstein, D.T., Daniel, J.C., & Springett, B.P. (2004) A test of the multi-predator hypothesis: Rapid loss of antipredator behavior after 130 years of isolation. *Ethology*, **110**, 919-934.

Boege, K. & Marquis, R.J. (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology & Evolution*, **20**, 441-448.

Bolnick, D.I., Amarasekare, P., Araujo, M.S., Burger, R., Levine, J.M., Novak, M., Rudolf, V.H., Schreiber, S.J., Urban, M.C., & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183-192.

Bolnick, D.I. & Preisser, E.L. (2005) Resource competition modifies the strength of trait-mediated predator-prey interactions: A meta-analysis. *Ecology*, **86**, 2771-2779.

Bonamour, S., Teplitsky, C., Charmantier, A., Crochet, P.A., & Chevin, L.M. (2017) Selection on skewed characters and the paradox of stasis. *Evolution*, **71**, 2703-2713.

Bookstein, F.L. (1997) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge, UK.

Bourdeau, P.E., Butlin, R.K., Bronmark, C., Edgell, T.C., Hoverman, J.T., & Hollander, J. (2015) What can aquatic gastropods tell us about phenotypic plasticity? A review and meta-analysis. *Heredity*, **115**, 312-321.

Bourdeau, P.E. & Johansson, F. (2012) Predator-induced morphological defences as by-products of prey behaviour: a review and prospectus. *Oikos*, **121**, 1175-1190.

Brock, K.M., Bednekoff, P.A., Pafilis, P., & Foufopoulos, J. (2015) Evolution of antipredator behavior in an island lizard species, *Podarcis erhardii* (Reptilia: Lacertidae): The sum of all fears? *Evolution*, **69**, 216-31.

Brodie, E.D. (1999) Costs of exploiting poisonous prey: evolutionary trade-offs in a predator-prey arms race. *Evolution*, **53**, 626-631.

Brodin, T. & Johansson, F. (2004) Conflicting selection pressures on the growth/predation risk trade-off in a damselfly. *Ecology*, **85**, 2927-2932.

Brown, J.H., Marquet, P.A., & Taper, M.L. (1993) Evolution of body size: consequences of an energetic definition of fitness. *The American Naturalist*, **142**, 573-584.

Brown, J.M., McPeek, M.A., & May, M.L. (2000) A phylogenetic perspective on habitat shifts and diversity in the north American *Enallagma* damselflies. *Systematic Biology*, **49**, 697-712.

Brown, J.S. & Vincent, T.L. (1992) Organization of predator-prey communities as an evolutionary game. *Evolution*, **46**, 1269-1283.

Bybee, S., Córdoba-Aguilar, A., Duryea, M.C., Futahashi, R., Hansson, B., Lorenzo-Carballa, M.O., Schilder, R., Stoks, R., Suvorov, A., Svensson, E.I., Swaegers, J., Takahashi, Y., Watts, P.C., & Wellenreuther, M. (2016) Odonata (dragonflies and damselflies) as a bridge between ecology and evolutionary genomics. *Frontiers in Zoology*, **13**, 46.

Chandler, H.C., Gorman, T.A., & Haas, C.A. (2016) The effects of crayfish predation and vegetation cover on tadpole growth, survival, and nonlethal injury. *Journal of Herpetology*, **50**, 271-277.

Chivers, D.P., Wisenden, B.D., & Smith, R.J.F. (1996) Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour*, **52**, 315-320.

Claessen, D., Van Oss, C., de Roos, A.M., & Persson, L. (2002) The impact of size-dependent predation on population dynamics and individual life history. *Ecology*, **83**, 1660-1675.

Claramunt, R.M. & Wahl, D.H. (2000) The effects of abiotic and biotic factors in determining larval fish growth rates: a comparison across species and reservoirs. *Transactions of the American Fisheries Society*, **129**, 835-851.

Clinchy, M., Sheriff, M.J., & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear. *Functional Ecology*, **27**, 56-65.

Corbet, P.S. (1999) Dragonflies: behaviour and ecology of Odonata. Comstock Publishing Associates, Ithaca, USA.

Coyne, J.A. & Orr, H.A. (2004) Speciation. Sinauer Associates, Inc., Sunderland, MA.

Crispo, E. (2007) The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution*, **61**, 2469-2479.

D'Agostino, R.B. (1970) Transformation to normality of the null distribution of g1. *Biometrika*, 679-681.

Dahl, J. & Peckarsky, B.L. (2002) Induced morphological defenses in the wild: predator effects on a mayfly, *Drunella coloradensis*. *Ecology*, **83**, 1620-1634.

Davenport, J.M., Hossack, B.R., & Lowe, W.H. (2014) Partitioning the non-consumptive effects of predators on prey with complex life histories. *Oecologia*, **176**, 149-155.

Dayton, G.H., Saenz, D., Baum, K.A., Langerhans, R.B., & DeWitt, T.J. (2005) Body shape, burst speed and escape behavior of larval anurans. *Oikos*, **111**, 582-591.

De Block, M., Slos, S., Johansson, F., & Stoks, R. (2008) Integrating life history and physiology to understand latitudinal size variation in a damselfly. *Ecography*, **31**, 115-123.

De Block, M. & Stoks, R. (2003) Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *Journal of Evolutionary Biology*, **16**, 986-95.

Denver, R.J. (2009) Stress hormones mediate environment-genotype interactions during amphibian development. *General and Comparative Endocrinology*, **164**, 20-31.

DeWitt, T.J., Robinson, B.W., & Wilson, D.S. (2000) Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evolutionary Ecology Research*, **2**, 129-148.

Dewitt, T.J., Sih, A., & Hucko, J.A. (1999) Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Animal Behaviour*, **58**, 397-407.

Dijkstra, K.D., Monaghan, M.T., & Pauls, S.U. (2014) Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology*, **59**, 143-63.

Dmitriew, C.M. (2011) The evolution of growth trajectories: what limits growth rate? *Biological Reviews*, **86**, 97-116.

Dudgeon, D. (1989) Life cycle, production, microdistribution and diet of the damselfly *Euphaea decorata* (Odonata: Euphaeidae) in a Hong Kong forest stream. *Journal of Zoology*, **217**, 57-72.

Edeline, E., Ari, T.B., Vøllestad, L.A., Winfield, I.J., Fletcher, J.M., James, J.B., & Stenseth, N.C. (2008) Antagonistic selection from predators and pathogens alters food-web structure. *Proceedings of the National Academy of Sciences*, **105**, 19792-19796.

Edgell, T.C., Lynch, B.R., Trussell, G.C., & Palmer, A.R. (2009) Experimental evidence for the rapid evolution of behavioral canalization in natural populations. *The American Naturalist*, **174**, 434-440.

Edmunds, M. (1974) Defence in animals: a survey of anti-predator defences. Longman, Harlow.

Egea-Serrano, A., Hangartner, S., Laurila, A., & Räsänen, K. (2014) Multifarious selection through environmental change: acidity and predator-mediated adaptive divergence in the moor frog (*Rana arvalis*). *Proceedings of the Royal Society B: Biological Sciences*, **281**.

Ehlinger, T.J. (1989) Learning and individual variation in bluegill foraging: habitat-specific techniques. *Animal Behaviour*, **38**, 643-658.

Eklöv, P. & Werner, E.E. (2000) Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. *Oikos*, **88**, 250-258.

Emerson, B.C. (2008) Speciation on islands: what are we learning? *Biological Journal of the Linnean Society*, **95**, 47-52.

Ercit, K. (2016) Temporal variation in selection on male and female traits in wild tree crickets. *Ecology and evolution*, **6**, 5118-5128.

Eroukhmanoff, F. & Svensson, E.I. (2009) Contemporary parallel diversification, antipredator adaptations and phenotypic integration in an aquatic isopod. *PLoS One*, **4**, e6173.

Falck, J. & Johansson, F. (2000) Patterns in size, sex ratio and time at emergence in a south Swedish population of *Sympetrum sanguineum* (Odonata). *Aquatic Insects*, **22**, 311-317.

Felsenstein, J. (1985) Phylogenies and the Comparative Method. *The American Naturalist*, **125**, 1-15.

Ferrari, M.C.O., Sih, A., & Chivers, D.P. (2009) The paradox of risk allocation: a review and prospectus. *Animal Behaviour*, **78**, 579-585.

Flenner, I.D.A., Olne, K., Suhling, F., & SahlÉN, G. (2009) Predator-induced spine length and exocuticle thickness in *Leucorrhinia dubia* (Insecta: Odonata): a simple physiological trade-off? *Ecological Entomology*, **34**, 735-740.

Franks, D.W. & Oxford, G.S. (2017) The co-evolution of anti-predator polymorphisms in sympatric populations. *Biological Journal of the Linnean Society*, **122**, 729-737.

Freckleton, R.P., Harvey, P.H., & Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist*, **160**, 712-726.

Gaedke, U. & Klauschies, T. (2017) Analyzing the shape of observed trait distributions enables a data-based moment closure of aggregate models. *Limnology and Oceanography: Methods*, **15**, 979-994.

Garamszegi, L.Z. & Moller, A.P. (2017) Partitioning within-species variance in behaviour to within- and between-population components for understanding evolution. *Ecology Letters*, **20**, 599-608.

Garland, T., Bennett, A.F., & Rezende, E.L. (2005) Phylogenetic approaches in comparative physiology. *Journal of experimental Biology*, **208**, 3015-3035.

Gerritsen, J. & Strickler, J.R. (1977) Encounter probabilities and community structure in zooplankton: a mathematical model. *Journal of the Fisheries Board of Canada*, **34**, 73-82.

Gilinsky, E. (1984) The role of fish predation and spatial heterogeneity in determining benthic community structure. *Ecology*, **65**, 455-468.

Gosden, T.P., Stoks, R., & Svensson, E.I. (2011) Range limits, large-scale biogeographic variation, and localized evolutionary dynamics in a polymorphic damselfly. *Biological Journal of the Linnean Society*, **102**, 775-785.

Gosden, T.P., Waller, J.T., & Svensson, E.I. (2015) Asymmetric isolating barriers between different microclimatic environments caused by low immigrant survival. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 2014.2459.

Gotthard, K., Nylin, S., & Wiklund, C. (1994) Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, Pararge aegeria. *Oecologia*, **99**, 281-289.

Gurevitch, J. & Padilla, D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, **19**, 470-474.

Hélène, A.C., Wilfried, T., Gilles, Y.N., Rolland, D., Serge, A., & Sandra, L. (2010) A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology*, **24**, 1192-1201.

Hambright, K.D. (1991) Experimental analysis of prey selection by largemouth bass: role of predator mouth width and prey body depth. *Transactions of the American Fisheries Society*, **120**, 500-508.

Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E., & Challenger, W. (2008) GEIGER: investigating evolutionary radiations. *Bioinformatics*, **24**, 129-131.

Harris, S., Eroukhmanoff, F., Green, K.K., Svensson, E.I., & Pettersson, L.B. (2011) Changes in behavioural trait integration following rapid ecotype divergence in an aquatic isopod. *Journal of Evolutionary Biology*, **24**, 1887-1896.

Harvey, H.H. & McArdle, J.M. (1987). Composition of the Benthos in Relation to pH in the LaCloche Lakes. In Acidic Precipitation: Proceedings of the International Symposium on Acidic Precipitation Muskoka, Ontario, September 15–20, 1985 (ed. by H.C. Martin), pp. 529-536. Springer Netherlands, Dordrecht, Netherlands.

Harvey, P.H., Read, A.F., & Nee, S. (1995) Why ecologists need to be phylogenetically challenged. *Journal of Ecology*, **83**, 535-536.

Hawlena, D. & Schmitz, O.J. (2010a) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences*, **107**, 15503-15507.

Hawlena, D. & Schmitz, O.J. (2010b) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist*, **176**, 537-556.

Henrikson, B.I. (1993) Sphagnum mosses as a microhabitat for invertebrates in acidified lakes and the color adaptation and substrate preference in *Leucorrhinia dubia* (Odonata, Anisoptera). *Ecography*, **16**, 143-153.

Herberholz, J. & Marquart, G.D. (2012) Decision Making and Behavioral Choice during Predator Avoidance. *Frontiers in Neuroscience*, **6**, 125.

Herczeg, G., Gonda, A., & Merilä, J. (2009) Predation mediated population divergence in complex behaviour of nine-spined stickleback (*Pungitius pungitius*). *Journal of evolutionary biology*, **22**, 544-552.

Higham, T.E., Rogers, S.M., Langerhans, R.B., Jamniczky, H.A., Lauder, G.V., Stewart, W.J., Martin, C.H., & Reznick, D.N. (2016) Speciation through the lens of biomechanics: locomotion, prey capture and reproductive isolation. *Proceedings of the Royal Society B: Biological Sciences*, **283**.

Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theoretical population biology*, **12**, 197-229.

Honěk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 483-492.

Hoso, M., Kameda, Y., Wu, S.P., Asami, T., Kato, M., & Hori, M. (2010) A speciation gene for left-right reversal in snails results in anti-predator adaptation. *Nature Communication*, **1**, 133.

Hoverman, J.T., Auld, J.R., & Relyea, R.A. (2005) Putting prey back together again: integrating predator-induced behavior, morphology, and life history. *Oecologia*, **144**, 481-491.

Hoverman, J.T. & Relyea, R.A. (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology*, **88**, 693-705.

Hovmöller, R. & Johansson, F. (2004) A phylogenetic perspective on larval spine morphology in *Leucorrhinia* (Odonata: Libellulidae) based on ITS1, 5.8S, and ITS2 rDNA sequences. *Molecular Phylogenetics and Evolution*, **30**, 653-662.

Hughes, M. (1966) Patters of ventilation in dragonfly larvae. *Journal of Experimental Biology*, **52**, 167-175.

Ingley, S.J., Billman, E.J., Belk, M.C., & Johnson, J.B. (2014) Morphological divergence driven by predation environment within and between species of *Brachyrhaphis* fishes. *PloS one*, **9**, e90274.

Janssens, L. & Stoks, R. (2013) Predation risk causes oxidative damage in prey. *Biology Letters*, **9**.

Januszkiewicz, A.J. & Robinson, B.W. (2007) Divergent walleye (Sander vitreus)-mediated inducible defenses in the centrarchid pumpkinseed sunfish (*Lepomis gibbosus*). *Biological Journal of the Linnean Society*, **90**, 25-36.

Jeschke, J.M. (2006) Density-dependent effects of prey defenses and predator offenses. *Journal of Theoretical Biology*, **242**, 900-907.

Jiang, B. & Mikolajewski, D.J. (2018) Shift in predation regime mediates diversification of foraging behaviour in a dragonfly genus. *Ecological Entomology*, **43**,525-533.

Jobling, M. (1983) Effect of feeding frequency on food intake and growth of Arctic charr, *Salvelinus alpinus* L. *Journal of Fish Biology*, **23**, 177-185.

Johansson, F. (2002) Reaction norms and production costs of predator-induced morphological defences in a larval dragonfly (*Leucorrhinia dubia*: Odonata). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **80**, 944-950.

Johansson, F. & Andersson, J. (2009) Scared fish get lazy, and lazy fish get fat. *Journal of Animal Ecology*, **78**, 772-777.

Johansson, F. & Brodin, T. (2003) Effects of fish predators and abiotic factors on dragonfly community structure. *Journal of Freshwater Ecology*, **18**, 415-423.

Johansson, F., Halvarsson, P., Mikolajewski, D., & Höglund, J. (2017) Genetic differentiation in the boreal dragonfly *Leucorrhinia dubia* in the Palearctic region. *Biological Journal of the Linnean Society*, **121**, 294-304.

Johansson, F. & Mikolajewski, D.J. (2008). Evolution of morphological defences. In Dragonflies and damselflies: model organisms for ecological and evolutionary research (ed. Cordoba-Aguilar A.), pp. 127-139. Oxford University Press, New York.

Johansson, F. & Samuelsson, L. (1994) Fish-induced variation in abdominal spine length of *Leucorrhinia dubia* (Odonata) larvae? *Oecologia*, **100**, 74-79.

Johansson, F. & Stoks, R. (2005) Adaptive plasticity in response to predators in dragonfly larvae and other aquatic insects. *Insect evolutionary ecology. CABI Publishing, Walford*, 347-370.

Johnson, D.M. (1991) Behavioral ecology of larval dragonflies and damselflies. *Trends in Ecology & Evolution*, **6**, 8-13.

Johnson, P.T., Preu, E.R., Sutherland, D.R., Romansic, J.M., Han, B., & Blaustein, A.R. (2006) Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. *Ecology*, **87**, 2227-2235.

Jones, A.G., Bürger, R., Arnold, S.J., Hohenlohe, P.A., & Uyeda, J.C. (2012) The effects of stochastic and episodic movement of the optimum on the evolution of the G-matrix and the response of the trait mean to selection. *Journal of evolutionary biology*, **25**, 2210-2231.

Katz, S.M., Pollock, F.J., Bourne, D.G., & Willis, B.L. (2014) Crown-of-thorns starfish predation and physical injuries promote brown band disease on corals. *Coral Reefs*, **33**, 705-716.

Kemppainen, P., Lindskog, T., Butlin, R., & Johannesson, K. (2011) Intron sequences of arginine kinase in an intertidal snail suggest an ecotype-specific selective sweep and a gene duplication. *Heredity*, **106**, 808.

Kerfoot, W.C. & Sih, A. (1987) Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, PA.

Kim, S.Y. & Velando, A. (2015) Phenotypic integration between antipredator behavior and camouflage pattern in juvenile sticklebacks. *Evolution*, **69**, 830-838.

Kingsolver, J.G. & Diamond, S.E. (2011) Phenotypic selection in natural populations: what limits directional selection? *The American Naturalist*, **177**, 346-357.

Kingsolver, J.G., Diamond, S.E., Siepielski, A.M., & Carlson, S.M. (2012) Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary Ecology*, **26**, 1101-1118.

Kishida, O., Trussell, G.C., Mougi, A., & Nishimura, K. (2009) Evolutionary ecology of inducible morphological plasticity in predator-prey interaction: toward the practical links with population ecology. *Population Ecology*, **52**, 37-46.

Klingenberg, C.P. (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular ecology resources*, **11**, 353-357.

Klingenberg, C.P., Barluenga, M., & Meyer, A. (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution*, **56**, 1909-1920.

Komsta, L. & Novomestky, F. (2015) Moments, cumulants, skewness, kurtosis and related tests. *R package version 0.14*.

Koons, D.N., Metcalf, C.J.E., & Tuljapurkar, S. (2008) Evolution of delayed reproduction in uncertain environments: a life-history perspective. *The American Naturalist*, **172**, 797-805.

Kucharski, R. & Maleszka, R. (1998) Arginine kinase is highly expressed in the compound eye of the honey-bee, Apis mellifera. *Gene*, **211**, 343-349.

Labra, A. & Niemeyer, H.M. (2004) Variability in the assessment of snake predation risk by *Liolaemus* lizards. *Ethology*, **110**, 649-662.

Lagergren, R., Hellsten, M., & Stenson, J. (1997) Increased drag, and thus lower speed: a cost for morphological defence in *Bosmina* (*Eubosmina*) (Crustacea: Cladocera). *Functional Ecology*, **11**, 484-488.

Lagergren, R., Lord, H., & Stenson, J.A.E. (2001) Influence of temperature on hydrodynamic costs of morphological defences in zooplankton: experiments on models of *Eubosmina* (Cladocera). *Functional Ecology*, **14**, 380-387.

Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T., Coss, R.G., Donohue, K., & Foster, S.A. (2009) Relaxed selection in the wild. *Trends in ecology & evolution*, **24**, 487-496.

Lande, R. & Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210-1226.

Langerhans, R.B. (2007). Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. Predation in organisms, pp. 177-220. Springer, Heidelberg , Germany.

Langerhans, R.B. (2009) Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology*, **22**, 1057-1075.

Langerhans, R.B. & DeWitt, T.J. (2004) Shared and unique features of evolutionary diversification. *The American Naturalist*, **164**, 335-349.

Lass, S. & Spaak, P. (2003) Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia*, **491**, 221-239.

Laurila, A., Järvi-Laturi, M., Pakkasmaa, S., & Merilä, J. (2004) Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. *Oikos*, **107**, 90-99.

Laurila, A., Pakkasmaa, S., & Merila, J. (2006) Population divergence in growth rate and antipredator defences in Rana arvalis. *Oecologia*, **147**, 585-595.

Lenth, R. (2018) Emmeans: Estimated marginal means, aka least-squares means. *R Package Version*, **1**.

Lenth, R. & Lenth, M.R. (2016) Package 'lsmeans'. Avialable at: cran.r-project.org/ web/packages/lsmeans.

Lima, S.L. & Dill, L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619-640.

Lind, J. (2005) Determining the fitness consequences of antipredation behavior. *Behavioral Ecology*, **16**, 945-956.

Losos, J.B., Mouton, P.L.F.N., Bickel, R., Cornelius, I., & Ruddock, L. (2002) The effect of body armature on escape behaviour in cordylid lizards. *Animal Behaviour*, **64**, 313-321.

Losos, J.B., Schoener, T.W., & Spiller, D.A. (2004) Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. *Nature*, **432**, 505-508.

Lundvall, D., Svanbäck, R., Persson, L., & Byström, P. (1999) Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1285-1292.

Márquez, E. & Knowles, L.L. (2007) Correlated evolution of multivariate traits: detecting co-divergence across multiple dimensions. *Journal of Evolutionary Biology*, **20**, 2334-2348.

Ma, X., Wolinska, J., Petrusek, A., Gießler, S., Hu, W., & Yin, M. (2016) The phenotypic plasticity in Chinese populations of *Daphnia similoides sinensis:* recurvate helmeted forms are associated with the presence of predators. *Journal of Plankton Research*, **38**, 855-864.

MacLeod, K.J., Krebs, C.J., Boonstra, R., & Sheriff, M.J. (2018) Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. *Oikos*, **127**, 375-380.

Magalhaes, I.S., D'Agostino, D., Hohenlohe, P.A., & MacColl, A.D. (2016) The ecology of an adaptive radiation of three-spined stickleback from North Uist, Scotland. *Molecular Ecology*, **25**, 4319-4336.

Mangel, M. & Stamps, J. (2001) Trade-offs between growth and mortality and the maintenance of individual variation in growth. *Evolutionary Ecology Research,* **3**.

Marchinko, K.B. (2009) Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. *Evolution: International Journal of Organic Evolution*, **63**, 127-138.

Marden, J.H. (1987) Maximum lift production during takeoff in flying animals. *Journal of experimental Biology*, **130**, 235-258.

Mardia, K.V., Bookstein, F.L., & Moreton, I.J. (2000) Statistical assessment of bilateral symmetry of shapes. *Biometrika*, **87**, 285-300.

Martin, R.A., Riesch, R., Heinen-Kay, J.L., & Langerhans, R.B. (2014) Evolution of male coloration during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). *Evolution*, **68**, 397-411.

McCauley, S.J. (2006) The effects of dispersal and recruitment limitation on community structure of odonates in artificial ponds. *Ecography*, **29**, 585-595.

McCauley, S.J., Davis, C.J., & Werner, E.E. (2008) Predator induction of spine length in larval *Leucorrhinia intacta* (Odonata). *Evolutionary Ecology Research*, **10**, 435-447.

McCay, C.M., Crowell, M.F., & Maynard, L.A. (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *The journal of Nutrition*, **10**, 63-79.

McCollum, S.A. & Leimberger, J.D. (1997) Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia*, **109**, 615-621.

McPeek, M.A. (1990a) Behavioral-Differences between *Enallagma* Species (Odonata) Influencing Differential Vulnerability to Predators. *Ecology,* **71**, 1714-1726.

McPeek, M.A. (1990b) Determination of Species Composition in the *Enallagma* Damselfly Assemblages of Permanent Lakes. *Ecology*, **71**, 83-98.

McPeek, M.A. (1995) Morphological Evolution Mediated by Behavior in the Damselflies of two Communities. *Evolution*, **49**, 749-769.

McPeek, M.A. (1999) Biochemical evolution associated with antipredator adaptation in damselflies. *Evolution*, **53**, 1835-1845.

McPeek, M.A. (2004) The growth/predation risk trade-off: So what is the mechanism? *American Naturalist*, **163**, E88-E111.

McPeek, M.A., Grace, M., & Richardson, J.M. (2001) Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. *Ecology*, **82**, 1535-1545.

McPeek, M.A., Schrot, A.K., & Brown, J.M. (1996) Adaptation to predators in a new community: Swimming performance and predator avoidance in damselflies. *Ecology*, **77**, 617-629.

Mennen, G.J. & Laskowski, K.L. (2018) Defence is the best offence: invasive prey behaviour is more important than native predator behaviour. *Animal Behaviour*, **138**, 157-164.

Miehls, A.L., Peacor, S.D., & McAdam, A.G. (2014) Gape-limited predators as agents of selection on the defensive morphology of an invasive invertebrate. *Evolution*, **68**, 2633-2643.

Mikolajewski, D.J., De Block, M., Rolff, J., Johansson, F., Beckerman, A.P., & Stoks, R. (2010) Predator-driven trait diversification in a dragonfly genus: covariation in behavioral and morphological antipredator defense. *Evolution*, **64**, 3327-3325.

Mikolajewski, D.J., De Block, M., & Stoks, R. (2015a) The interplay of adult and larval time constraints shapes species differences in larval life history. *Ecology*, **96**, 1128-1138.

Mikolajewski, D.J. & Johansson, F. (2004) Morphological and behavioral defenses in dragonfly larvae: trait compensation and cospecialization. *Behavioral Ecology*, **15**, 614-620.

Mikolajewski, D.J., Johansson, F., Wohlfahrt, B., & Stoks, R. (2006) Invertebrate predation selects for the loss of a morphological antipredator trait. *Evolution*, **60**, 1306-1310.

Mikolajewski, D.J. & Rolff, J. (2004) Benefits of morphological defence demonstrated by direct manipulation in larval dragonflies. *Evolutionary Ecology Research*, **6**, 619-626.

Mikolajewski, D.J., Rusen, L., Mauersberger, R., Johansson, F., & Rolff, J. (2015b) Relaxed predation results in reduced phenotypic integration in a suite of dragonflies. *Journal of Evolutionary Biology*, **28**, 1354-1363.

Mikolajewski, D.J., Scharnweber, K., Jiang, B., Leicht, S., Mauersberger, R., & Johansson, F. (2016) Changing the habitat: the evolution of intercorrelated traits to escape from predators. *Journal of evolutionary biology*, **29**, 1394-1405.

Mikolajewski, D.J., Wohlfahrt, B., Joop, G., & Beckerman, A.P. (2013) Sexual size dimorphism and the integration of phenotypically plastic traits. *Ecological Entomology*, **38**, 418-428.

Mill, P. & Hughes, G. (1966) The nervous control of ventilation in dragonfly larvae. *Journal of Experimental Biology*, **44**, 297-316.

Mill, P.J. & Pickard, R.S. (1975) Jet-Propulsion in Anisopteran Dragonfly Larvae. *Journal of Comparative Physiology*, **97**, 329-338.

Mitchell, M.D., Bairos-Novak, K.R., & Ferrari, M.C.O. (2017) Mechanisms underlying the control of responses to predator odours in aquatic prey. *Journal of Experimental Biology*, **220**, 1937-1946.

Moczek, A.P. & Nijhout, H.F. (2002) Developmental mechanisms of threshold evolution in a polyphenic beetle. *Evolution & Development*, **4**, 252-264.

Moodie, G. (1972) Predation, natural selection and adaptation in an unusual threespine stickleback. *Heredity*, **28**, 155.

Morin, P.J. (1985) Predation intensity, prey survival and injury frequency in an amphibian predator-prey interaction. *Copeia*, 638-644.

Munch, S.B. & Conover, D.O. (2003) Rapid growth results in increased susceptibility to predation in *Menidia menidia*. *Evolution*, **57**, 2119-2127.

Nakagawa, S., Poulin, R., Mengersen, K., Reinhold, K., Engqvist, L., Lagisz, M., Senior, A.M., & O'Hara, R.B. (2015) Meta-analysis of variation: ecological and evolutionary applications and beyond. *Methods in Ecology and Evolution*, **6**, 143-152.

Niemelä, P.T., Vainikka, A., Hedrick, A.V., & Kortet, R. (2012) Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Functional Ecology*, **26**, 450-456.

Nijhout, H.F. & Emlen, D.J. (1998) Competition among body parts in the development and evolution of insect morphology. *Proceedings of the National Academy of Sciences*, **95**, 3685-3689.

Nijhout, H.F. & German, R.Z. (2012) Developmental causes of allometry: new models and implications for phenotypic plasticity and evolution. *Integrative and Comparative Biology*, **52**, 43-52.

Nilsson, P.A. & Brönmark, C. (2000) Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos*, **88**, 539-546.

Nosil, P. & Crespi, B.J. (2006) Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences*, **103**, 9090-9095.

Nunes, A.L., Orizaola, G., Laurila, A., & Rebelo, R. (2014) Morphological and life-history responses of anurans to predation by an invasive crayfish: an integrative approach. *Ecology and Evolution*, **4**, 1491-503.

O'Steen, S., Cullum, A.J., & Bennett, A.F. (2002) Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **56**, 776-784.

Orme, D. (2013) The caper package: comparative analysis of phylogenetics and evolution in R. *R package version*, **5**, 1-36.

Oufiero, C.E., Walsh, M.R., Reznick, D.N., & Garland Jr, T. (2011) Swimming performance trade-offs across a gradient in community composition in Trinidadian killifish (*Rivulus hartii*). *Ecology*, **92**, 170-179.

Pafilis, P., Foufopoulos, J., Poulakakis, N., Lymberakis, P., & Valakos, E.D. (2009) Tail shedding in island lizards [Lacertidae, Reptilia]: decline of antipredator defenses in relaxed predation environments. *Evolution*, **63**, 1262-1278.

Pagel, M.D. & Harvey, P.H. (1989) Comparative methods for examining adaptation depend on evolutionary models. *Folia Primatologica*, **53**, 203-220.

Paine, C.E.T., Marthews, T.R., Vogt, D.R., Purves, D., Rees, M., Hector, A., & Turnbull, L.A. (2012) How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution*, **3**, 245-256.

Pajunen, V.I. (1962) Studies on the population ecology of *Leucorrhinia dubia* vd Lind.(Odon., Libellulidae). *Annales Zoologici Societatis Zoologicae-Botanicae Fennicae 'Vanamo'*, **24**, 1–79.

Peacor, S.D. & Pfister, C.A. (2006) Experimental and model analyses of the effects of competition on individual size variation in wood frog (*Rana sylvatica*) tadpoles. *Journal of Animal Ecology*, **75**, 990-999.

Peacor, S.D., Schiesari, L., & Werner, E.E. (2007) Mechanisms of nonlethal predator effect on cohort size variation: ecological and evolutionary implications. *Ecology*, **88**, 1536-1547.

Peckarsky, B.L. & McIntosh, A.R. (1998) Fitness and community consequences of avoiding multiple predators. *Oecologia*, **113**, 565-576.

Peckarsky, B.L., Taylor, B.W., McIntosh, A.R., McPeek, M.A., & Lytle, D.A. (2001) Variation in mayfly size at metamorphosis as a developmental response to risk of predation. *Ecology*, **82**, 740-757.

Peters, R.H. & Peters, R.H. (1986) The ecological implications of body size. Cambridge University Press, Cambridge, UK.

Petrin, Z., Schilling, E.G., Loftin, C.S., & Johansson, F. (2010) Predators shape distribution and promote diversification of morphological defenses in *Leucorrhinia*, Odonata. *Evolutionary ecology*, **24**, 1003-1016.

Pettorelli, N., Hilborn, A., Duncan, C., & Durant, S.M. (2015). Individual variability: the missing component to our understanding of predator-prey interactions. In Advances in Ecological Research (ed. by S. Pawar, G. Woodward & A.I. Dell), Vol. 52, pp. 19-44. Academic Press.

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D., & Moczek, A.P. (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, **25**, 459-67.

Pigliucci, M. (2001) Phenotypic plasticity: beyond nature and nurture. JHU Press, Baltimore, USA.

Pigliucci, M. & Preston, K. (2004) Phenotypic integration. Oxford University Press, Oxford, UK.

Plckard, R. & Mill, P. (1974) Ventilatory movements of the abdomen and branchial apparatus in dragonfly larvae (Odonata: Anisoptera). *Journal of Zoology*, **174**, 23-40.

Poléo, A., Osxnevad, S.A., Össtbye, K., Heibo, E., Andersen, R.A., & Vøllestad, L.A. (1995) Body morphology of crucian carp *Carassius carassius* in lakes with or without piscivorous fish. *Ecography*, **18**, 225-229.

Price, S., Friedman, S., & Wainwright, P. (2015) How predation shaped fish: the impact of fin spines on body form evolution across teleosts. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 2015.1428.

Pujol-Buxó, E., Garriga, N., Richter-Boix, A., & Llorente, G.A. (2016) Growth strategies of tadpoles along the pond permanency gradient. *Evolutionary Ecology*, **30**, 1117-1132.

Reimchen, T.E. & Nosil, P. (2004) Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. *Evolution*, **58**, 1274-1281.

Relyea, R.A. (2001a) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, **82**, 523-540.

Relyea, R.A. (2001b) The relationship between predation risk and antipredator responses in larval anurans. *Ecology*, **82**, 541-554.

Relyea, R.A. (2003) How prey respond to combined predators: a review and an empirical test. *Ecology*, **84**, 1827-1839.

Relyea, R.A. (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology*, **85**, 172-179.

Revell, L.J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217-223.

Revelle, W. (2016) psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, http://CRAN.R-project.org/ package=psych Version = 1.6.4.

Reznick, D. (1982) The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. *Evolution*, **36**, 1236-1250.

Reznick, D. (1985) Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, 257-267.

Reznick, D. & Endler, J.A. (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia Reticulata*). *Evolution*, **36**, 160-177.

Reznick, D.N., Ghalambor, C.K., & Crooks, K. (2008) Experimental studies of evolution in guppies: a model for understanding the evolutionary consequences of predator removal in natural communities. *Molecular Ecology*, **17**, 97-107.

Richardson, J.M.L. (2001) A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behavioral Ecology*, **12**, 51-58.

Richardson, J.M.L. (2002) Burst swim speed in tadpoles inhabiting ponds with different top predators. *Evolutionary Ecology Research*, **4**, 627-642.

Robinson, B.W., Wilson, D.S., & Shea, G.O. (1996) Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes. *Ecology*, **77**, 170-178.

Rodd, F.H. & Reznick, D.N. (1997) Variation in the demography of guppy populations: The importance of predation and life histories. *Ecology,* **78**, 405-418.

Rohlf, F.J. & Slice, D. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, **39**, 40-59.

Rousset, F. (2017) An introduction to the spaMM package for mixed models.

Rundle, H.D. & Nosil, P. (2005) Ecological speciation. *Ecology letters*, **8**, 336-352.

Rundle, H.D., Vamosi, S.M., & Schluter, D. (2003) Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. *Proceedings of the National Academy of Sciences*, **100**, 14943-8.

Runemark, A., Brydegaard, M., & Svensson, E. (2014) Does relaxed predation drive phenotypic divergence among insular populations? *Journal of evolutionary biology*, **27**, 1676-1690.

Rychła, A., Benndorf, J., & Buczyński, P. (2011) Impact of pH and conductivity on species richness and community structure of dragonflies (Odonata) in small mining lakes. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, **179**, 41-50.

Scales, J.A., King, A.A., & Butler, M.A. (2009) Running for your life or running for your dinner: what drives fiber-type evolution in lizard locomotor muscles? *The American Naturalist*, **173**, 543-553.

Scharnweber, K., Watanabe, K., Syväranta, J., Wanke, T., Monaghan, M.T., & Mehner, T. (2013) Effects of predation pressure and resource use on morphological divergence in omnivorous prey fish. *BMC evolutionary biology*, **13**, 132.

Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual review of ecology and systematics*, 35-68.

Schell, V.A. & Kerekes, J.J. (1989) Distribution, abundance and biomass of benthic macroinvertebrates relative to pH and nutrients in eight lakes of Nova Scotia, Canada. *Water, Air, & Soil Pollution*, **46**, 359-374.

Schlichting, C.D. & Wund, M.A. (2014) Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. *Evolution*, **68**, 656-672.

Schluter, D. (2009) Evidence for ecological speciation and its alternative. *Science*, **323**, 737-41.

Schluter, D. & McPhail, J.D. (1992) Ecological character displacement and speciation in sticklebacks. *The American Naturalist*, **140**, 85-108.

Schmidt, B. & Van Buskirk, J. (2005) A comparative analysis of predator-induced plasticity in larval *Triturus* newts. *Journal of Evolutionary Biology*, **18**, 415-425.

Schmitz, O. (2017) Predator and prey functional traits: understanding the adaptive machinery driving predator-prey interactions. *F1000Res*, **6**, 1767.

Semple, S., Cowlishaw, G., & Bennett, P.M. (2002) Immune system evolution among anthropoid primates: parasites, injuries and predators. *Proceedings of the Royal Society of London B: Biological Sciences*, **269**, 1031-1037.

Shama, L.N., Campero-Paz, M., Wegner, K.M., De Block, M., & Stoks, R. (2011) Latitudinal and voltinism compensation shape thermal reaction norms for growth rate. *Molecular Ecology*, **20**, 2929-2941.

Sih, A. (1987) Predator and prey lifestyles: an evolutionary and ecological overview. *Predation: direct and indirect impacts on aquatic communities*, 203-224.

Sih, A. (2004). A behavioral ecological view of phenotypic plasticity. In Phenotypic Plasticity: Functional and Conceptual Approaches (DeWitt, TJ and Scheiner, SM, eds), pp. 112-126. Oxford University Press, New York, USA.

Sih, A., Crowley, P., Mcpeek, M., Petranka, J., & Strohmeier, K. (1985) Predation, Competition, and Prey Communities - a Review of Field Experiments. *Annual Review of Ecology and Systematics*, **16**, 269-311.

Skelly, D.K. (1992) Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology*, **73**, 704-708.

Slos, S. & Stoks, R. (2006) Behavioural correlations may cause partial support for the risk allocation hypothesis in damselfly larvae. *Ethology*, **112**, 143-151.

Slos, S. & Stoks, R. (2008) Predation risk induces stress proteins and reduces antioxidant defense. *Functional Ecology*, **22**, 637-642.

Sokolovska, N., Rowe, L., & Johansson, F. (2000) Fitness and body size in mature odonates. *Ecological Entomology*, **25**, 239-248.

Staaf, D.J., Gilly, W.F., & Denny, M.W. (2014) Aperture effects in squid jet propulsion. *Journal of Experimental Biology*, jeb. 082271.

Stephens, P. & Wiens, J. (2008) Testing for evolutionary trade-offs in a phylogenetic context: ecological diversification and evolution of locomotor performance in emydid turtles. *Journal of evolutionary biology*, **21**, 77-87.

Sternberg, K. & Buchwald, R. (1999) Die Libellen Baden-Württembergs Bd.1: Allgemeiner Teil: Kleinlibellen (Zygoptera), pp. 415-427. Ulmer, Stuttgart.

Sternberg, K. & Buchwald, R. (2000) Libellen Baden-Württembergs, Bd.2, Großlibellen (Anisoptera). Ulmer, Stuttgart, Germany.

Stevens, M. & Ruxton, G.D. (2012) Linking the evolution and form of warning coloration in nature. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 417-426.

Stoks, R., Block, M.D., & McPeek, M.A. (2006) Physiological costs of compensatory growth in a damselfly. *Ecology*, **87**, 1566-1574.

Stoks, R. & De Block, M. (2000) The influence of predator species and prey age on the immediate survival value of antipredator behaviours in a damselfly. *Archiv für Hydrobiologie*, **147**, 417-430.

Stoks, R., De Block, M., & McPeek, M.A. (2005) Alternative growth and energy storage responses to mortality threats in damselflies. *Ecology Letters*, **8**, 1307-1316.

Stoks, R., Govaert, L., Pauwels, K., Jansen, B., & De Meester, L. (2016) Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecology letters*, **19**, 180-190.

Stoks, R., McPeek, M., & Mitchell, J. (2003) Evolution of prey behavior in response to changes in predation regime: damselflies in fish and dragonfly lakes. *Evolution*, **57**, 574-585.

Stoks, R. & McPeek, M.A. (2003) Antipredator behavior and physiology determine *Lestes* species turnover along the pond-permanence gradient. *Ecology*, **84**, 3327-3338.

Stoks, R. & McPeek, M.A. (2006) A tale of two diversifications: reciprocal habitat shifts to fill ecological space along the pond permanence gradient. *The American Naturalist*, **168**, S50-72.

Stoks, R., Swillen, I., & De Block, M. (2012) Behaviour and physiology shape the growth accelerations associated with predation risk, high temperatures and southern latitudes in Ischnura damselfly larvae. *Journal of Animal Ecology*, **81**, 1034-40.

Strobbe, F., McPeek, M.A., De Block, M., & Stoks, R. (2010) Survival selection imposed by predation on a physiological trait underlying escape speed. *Functional ecology*, **24**, 1306-1312.

Stroud, J.T. & Losos, J.B. (2016) Ecological Opportunity and Adaptive Radiation. *Annual Review of Ecology, Evolution, and Systematics*, **47**, 507-532.

Suhling, F., Sahlen, G., Kasperski, J., & Gaedecke, D. (2005) Behavioural and life history traits in temporary and perennial waters: comparisons among three pairs of sibling dragonfly species. *Oikos*, **108**, 609-617.

Swaegers, J., Strobbe, F., McPeek, M., & Stoks, R. (2017) Selection on escape performance during ecological speciation driven by predation. *Animal behaviour*, **124**, 153-159.

Tan, E.J., Reid, C.A.M., Symonds, M.R.E., Jurado-Rivera, J.A., & Elgar, M.A. (2017) The Role of Life-History and Ecology in the Evolution of Color Patterns in Australian Chrysomeline Beetles. *Frontiers in Ecology and Evolution*, **5**, 140.

Thomaz, S.M. & Cunha, E.R.d. (2010) The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. *Acta Limnologica Brasiliensia*, **22**, 218-236.

Tigreros, N., Wang, E.H., & Thaler, J.S. (2018) Prey nutritional state drives divergent behavioural and physiological responses to predation risk. *Functional Ecology*, **32**, 982-989.

Tollrian, R. (1995) Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology*, 1691-1705.

Tollrian, R. & Harvell, C.D. (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton, NJ.

Tonn, W.M., Paszkowski, C.A., & Holopainen, I.J. (1992) Piscivory and recruitment: mechanisms structuring prey populations in small lakes. *Ecology*, **73**, 951-958.

Torres-Dowdall, J., Handelsman, C.A., Ruell, E.W., Auer, S.K., Reznick, D.N., & Ghalambor, C.K. (2012) Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies. *Functional Ecology*, **26**, 616-627.

Travis, J. (1989) The role of optimizing selection in natural populations. *Annual Review of Ecology and Systematics*, **20**, 279-296.

Travis, J., Keen, W.H., & Juilianna, J. (1985) The role of relative body size in a predator-prey relationship between dragonfly naiads and larval anurans. *Oikos*, 59-65.

Tregenza, T., Pritchard, V.L., & Butlin, R.K. (2000) Patterns of trait divergence between populations of the meadow grasshopper, Chorthippus parallelus. *Evolution*, **54**, 574-585.

Trueman, E. (1980) Swimming by jet propulsion. *Aspects of animal movement*, 93-105.

Uchmański, J. (1985) Differentiation and frequency distributions of body weights in plants and animals. *Philosophical Transactions of the Royal Society B*, **310**, 1-75.

Urban, M.C., Bürger, R., & Bolnick, D.I. (2013) Asymmetric selection and the evolution of extraordinary defences. *Nature communications*, **4**, 2085.

Vamosi, S.M. (2005) On the role of enemies in divergence and diversification of prey: a review and synthesis. *Canadian Journal of Zoology*, **83**, 894-910.

Vamosi, S.M. & Schluter, D. (2002) Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. *Proceedings of the Royal Society B-Biological Sciences*, **269**, 923-930.

Vamosi, S.M. & Schluter, D. (2004) Character shifts in the defensive armor of sympatric sticklebacks. *Evolution*, **58**, 376-385.

Van Dievel, M., Janssens, L., & Stoks, R. (2015) Short-and long-term behavioural, physiological and stoichiometric responses to predation risk indicate chronic stress and compensatory mechanisms. *Oecologia*, 1-11.

Vanhooydonck, B., Van Damme, R., & Aerts, P. (2001) Speed and stamina trade-off in lacertid lizards. *Evolution*, **55**, 1040-1048.

Vega-Trejo, R., Zúniga-Vega, J.J., & Langerhans, R.B. (2014) Morphological differentiation among populations of *Rhinella marina* (Amphibia: Anura) in western Mexico. *Evolutionary Ecology*, **28**, 69-88.

Vervust, B., Grbac, I., & Van Damme, R. (2007) Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. *Oikos*, **116**, 1343-1352.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V., & Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in ecology & evolution*, **27**, 244-252.

Voskarides, K. (2017) Plasticity vs Mutation. The role of microRNAs in human adaptation. *Mechanisms of Ageing and Development*, **163**, 36-39.

Walsh, M.R. & Reznick, D.N. (2010) Influence of the indirect effects of guppies on life-history evolution in *Rivulus hartii*. *Evolution*, **64**, 1583-1593.

Warren, P.H. & Lawton, J.H. (1987) Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? *Oecologia*, **74**, 231-235.

Wellborn, G.A. & Robinson, J.V. (1991) The influence of fish predation in an experienced prey community. *Canadian journal of zoology*, **69**, 2515-2522.

Wellborn, G.A., Skelly, D.K., & Werner, E.E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual review of ecology and systematics*, 337-363.

Wells, M. & O'Dor, R. (1991) Jet propulsion and the evolution of the cephalopods. *Bulletin of marine science*, **49**, 419-432.

Werner, E.E. & Anholt, B.R. (1993) Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist*, 242-272.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual review of Ecology and Systematics*, 249-278.

West-Eberhard, M.J. (2003) Developmental plasticity and evolution. Oxford University Press, Oxford, UK.

Westoby, M., Leishman, M., & Lord, J. (1995) Further remarks on phylogenetic correction. *Journal of Ecology*, **83**, 727-729.

Wheeler, D.E. (1991) The developmental basis of worker caste polymorphism in ants. *The American Naturalist*, **138**, 1218-1238.

Whittaker, R.J. & Fernández-Palacios, J.M. (2007) Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford, UK.

Wilbur, H.M. & Collins, J.P. (1973) Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science*, **182**, 1305-1314.

Wilson, E.A., Dudley, T.L., & Briggs, C.J. (2018) Shared behavioral responses and predation risk of anuran larvae and adults exposed to a novel predator. *Biological Invasions*, **20**, 475-485.

Wohlfahrt, B., Mikolajewski, D.J., Joop, G., & Suhling, F. (2006) Are behavioural traits in prey sensitive to the risk imposed by predatory fish? *Freshwater Biology*, **51**, 76-84.

Wund, M.A., Baker, J.A., Golub, J.L., & Foster, S.A. (2015) The evolution of antipredator behaviour following relaxed and reversed selection in Alaskan threespine stickleback fish. *Animal Behaviour*, **106**, 181-189.

Zanette, L.Y., White, A.F., Allen, M.C., & Clinchy, M. (2011) Perceived predation risk reduces the number of offspring songbirds produce per year. *Science*, **334**, 1398-1401.

Zera, A.J. & Denno, R.F. (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual review of entomology*, **42**, 207-230.

Ziemba, R.E., Myers, M.T., & Collins, J.P. (2000) Foraging under the risk of cannibalism leads to divergence in body size among tiger salamander larvae. *Oecologia*, **124**, 225-231.

Acknowledgements

Since I took something like dragonfly flying into Germany, I started to do research with dragonflies that are unlike airplanes. I vaguely know dragonflies came from water. In my village, we name this larval stage as “pfui xiaxia zi”, while adult dragonfly as “tingting”. I never tried to relate them together. But thanks to this project, I began to understand a tip of insect world iceberg, which I would like to know more in the future.

A lot of people I admired helped me in this study process. I would like to thank all of them with my deep gratitude. I would like to specially thank Dr. Dirk J. Mikolajewski. Thank you for your careful supervision on my work. You give me very valuable suggestions on my work, teach me how to work in the field and help me with my writings word by word. I am very grateful for that you helped me to adapt to new working environments at the beginning of my studies and guide me during the whole process of my studies. I would like also to thank Prof. Dr. Jens Rolff. Thank you for giving me opportunity to come to this group. You prepared me a friendly, international and harmonies study group. For every piece of my studies, you provided me constructive advice and enlightened me all the time. I am always impressed by your knowledge and from you I learned how to be a responsible group leader. Thank you for all supports to me in my study and life in Berlin. I would also to thank PD Dr. Renate Radek, who is always very friendly to me and help me a lot in my study and life. You are so patient that I always turn to you for help or advice when I meet some difficulties. During the cuticle measurements, you taught me how to use scanning electrical microscope. You provide valuable suggestions on this part of my studies. From you, I learned how to be a good teacher and colleague.

I would like show my deep gratitude to all my colleagues. From all of you, I gained power to finish my studies. We always discuss questions together, have fun together, and encourage each other. You become the reason that I always look forward to workdays. Therefore, I would like to thank Alex, Arpita, Baydaa, Beatriz, Caroline, Christin, Dino, Elisa, Greta, Guozhi, Janne, Lena, Luis, Maria, Olga, Paul, Pauline, Regina, Sabina, Shulin, Sophie, Thorben, Uta, Veronique. All the happy hours with you will be remembered by me

forever. Thank again for my colleges who helped me to read my early writing. They are Alex, Baydaa, Christin, Guozhi, Paul, and Sophie.

To successfully finish my studies, I received a lot help from the people or organization outside my group. I would like to thank Martin Friedrichs, Michael Hupfer, Justyna Wolinska from IGB and Anastasia Roberts (from AG Petermann) for providing me fish and equipment for some of my experiments. Martin also taught me how to keep the fish happy and alive. I would like to thank Frank Johansson from Uppsala University and Rüdiger Mauersberger from Förderverein Feldberg-Uckermärkische Seenlandschaft e.V. for providing samples to me. Frank also provided valuable suggestions on my research projects. I would like to thank China Scholarship Council for providing me stipend for all my study. Without this opportunity, I might have no chance to study abroad. I want to thank Anna Runemark (from Lund University) and Florian Ruland (from AG Jeschke) for discussing the analyses in chapter IV and Huidong Li (from Institute for Meteorology of FU) for helping me in doing figures.

In Berlin, I made a lot of friends. They are further sources to gain power. I would like to thank all my friends from FC Berlin volleyball team. With you I scream and laugh, which release all my pressures from work. I also must thank my friends from beach volleyball teams. With all of you, I explored another aspect of me. I want to show my gratitude to all my friends that came into Berlin with me together. We are so close that we are like sisters and brothers. We travelled together, cooked together, celebrated Chinese festival together. You are my family in Berlin. I also want to thank my friends from Wuhan botanical garden. They inspired me to go abroad and during these 4 years in Europe we have visited and encouraged each other a lot.

I would like thank my family’s support. They work very hard to support themselves and make me less worry about them. Thank my nieces for bring joy to the whole family. Thank my beloved grandma for her deep love to me.

Bin Jiang

29th, Jul. 2018, Berlin

List of publications

Bin Jiang, Dirk J. Mikolajewski. Shift in predation regime mediates diversification of foraging behaviour in a dragonfly genus. *Ecological Entomology*, 2018.

D. J. Mikolajewski, K. Scharnweber, **B. Jiang**, S. Leicht, R. Mauersberger, F. Johansson. Changing the habitat: the evolution of intercorrelated traits to escape from predators. *Journal of evolutionary biology*, 2016, 29(7), 1394-1405.

Bo Wang, **Bin Jiang**, Yuan Zhou, Yingjuan Su, Ting Wang. 2015. Higher substitution rates and lower dN/dS for the plastid genes in Gnetales than other gymnosperms. *Biochemical Systematics and Ecology*, 59: 278-287.

Bin Jiang, Lei Gao, Jia Li, Yuan zhou, Yingjuan Su, Ting Wang, Adaptive evolution of the chloroplast genome in AA-genome *Oryza* species, 2014 (20), *Chinese science bulletin*.

Conference activities

1) "Behavior choice of dragonfly *Leucorrhinia* species under different predation systems", 21st Annual Graduate Meeting of the DZG section Evolution Biology & Ecology, 6th-9th, March, 2016, Greifswald/Germany.

2) "Different Predators drive the Evolution of Anti-predator traits in a dragonfly genus", PhD student Meeting Bautzen 2017-Conflict and cooperation-bridging evolution, ecology and immunology, 16th-18th March 2017, Bautzen/Germany.

3) "Predation drives trait diversification", PhD meeting in animal evolutionary biology, 2-4 March, 2018, Svatý Jan pod Skalou/Czech Republic.

4) "Predation promotes diversification in mean and variance of antipredator traits ", XI European Congress of Entomology, 2-6 July, 2018, Naples/Italy.