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# Ecophysiology of the European spruce bark beetle (*Ips typographus* L.): Factors affecting individual fitness, dispersal and population dynamics

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*„Tief im Wald, zwischen Moos und Farn,*
*da lebte ein Käfer mit Namen Karl.*
*Sein Leben wurde jäh gestört,*
*als er ein dumpfes Grollen hört.*
*Lärmende Maschinen überrollen den Wald,*
*übertönen den Gesang der Vögel schon bald.*
*Mit scharfer Axt fällt man Baum um Baum,*
*zerstört damit seinen Lebensraum.*

*Karl der Käfer wurde nicht gefragt,*
*man hatte ihn einfach fortgejagt.“*

Gänsehaut (1983)

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# General introduction

Bark beetles (Coleoptera, Curculionidae, Scolytinae) are of fundamental importance for forest ecosystems. They are among the first in decomposing wood of dying or recently died trees and are an important rejuvenating agent in natural forests. By creating dead wood and forest gaps they facilitate fungal, insect and plant diversity (Müller et al., 2008; Müller et al., 2010; Wermelinger, 2004). The subfamily Scolytinae comprises wood boring species, the ambrosia beetles, which feed on associated fungi, and subcortical feeding species, the bark beetles (Kirkendall, 1983; Rudinsky, 1962). The Scolytinae include mono- and polyphagous species, which all have certain adaptations to their hosts regarding preference for tree age, bark thickness, branches, roots or cones. Most of the more than 5800 ambrosia and bark beetle species described worldwide (Wood and Bright Jr., 1992) prefer dead or weakened host trees in endemic conditions, and do not even attack healthy trees when population densities are high (Rudinsky, 1962). However, probably less than a dozen species, mainly in the genera *Dendroctonus* of America and *Ips* of America and Europe, can attack and kill vigorous trees when in epidemic population phases (Franceschi et al., 2005; Rudinsky, 1962). The small number of species capable of tree-killing might indicate a low functional redundancy (Müller et al., 2008) and thus highlights their importance for the ecosystem function they provide. Aggressive species can kill extensive areas of trees in epidemic conditions (Berryman, 1982), most remarkable in forests shaped by human activity. For example, the mountain pine beetle *Dendroctonus ponderosae* Hopkins killed 80 million pine trees between 1979 and 1983 (McGregor, 1985). It damaged 25 million ha of pine forests in the last decade alone (Bentz et al., 2010). The southern pine beetle *D. frontalis* Zimmerman killed more than 17 million $m^3$ pines in North America (Hoffard, 1985). In Europe, the spruce bark beetle *Ips typographus* Linneaus is the most aggressive scolytid species (Rudinsky, 1962). *I. typographus* larvae and imagines feed on the secondary phloem almost exclusively of Norway spruce *Picea abies* (L.) Karsten (Coniferales, Pinaceae) (Postner, 1974). Its distribution ranges across the whole of Europe as far as East Asia and is closely related to that of *P. abies* (Postner, 1974; Sallé et al., 2007). Apart from their important ecosystem function and complex population dynamics which make them generally interesting for ecologists and biologists alike, the damage bark beetles have caused in economically used forests in the last decades has facilitated extensive research.

*Life cycle and development*

With the exception of a short dispersal phase, all scolytid species have in common that they complete their whole life cycle cryptically: either under the bark, in the wood or in cones (Rudinsky, 1962). The life cycle is roughly divided into three phases, namely host tree colonisation, development and dispersal. In *I. typographus*, the life cycle begins in spring when overwintered adults disperse for locating suitable host trees. After host selection, males bore into the bark and excavate a mating chamber. There they mate with one to three females, in rare cases with more than three (Postner, 1974). Females then excavate egg galleries parallel to phloem fibres. Along the margins of these galleries they deposit up to 80 eggs in egg niches (Wermelinger, 2004). After egg deposition and a regeneration feeding, females may leave the host tree to initiate one or even more additional sister broods elsewhere in the same or in another tree (e.g. Anderbrant and Löfqvist, 1988). The larvae feed on the secondary phloem perpendicularly to egg galleries. Larval galleries end in a pupal chamber, which are then enlarged by eclosed callow beetles for maturation feeding before they emerge from their brood tree for dispersal (Postner, 1974). Development in *I. typographus* is highly temperature dependent. The average lower temperature threshold across all developmental stages is 8.3°C with an optimum of approximately 30°C. An accumulated temperature sum of 334 day degrees (dd) is required for completing development from egg to pupa and 239 dd are required for maturation feeding (Wermelinger and Seifert, 1998; 1999). The developmental temperature dependence leads to univoltine, bivoltine or even trivoltine populations, depending on latitude and elevation and thereby length of the season and accumulated temperature sum (Annila, 1969; Faccoli and Bernardinelli, 2011; Wermelinger et al., 2012; Wild, 1953).

*Population dynamics*

Characteristically for an r-strategist, *I. typographus* exploits scattered and ephemeral resources (i.e. weakened or dying trees) in endemic population phases. In contrast, populations can rapidly grow and beetles can colonise and kill vigorous spruces in epidemic phases (Mulock and Christiansen, 1986; Wermelinger, 2004). Switching from latency (endemic phase) to gradation (epidemic phase) is favoured by natural disturbances such as droughts, heat waves or storms (Wermelinger, 2004) which are expected to occur more frequently and more intensely in the twenty-first century (Luterbacher et al., 2007; Meehl and Tebaldi, 2004; Netherer and Schopf, 2010). The storms ‘Vivian’/‘Wiebke’ and ‘Lothar’ in the 1990s felled or uprooted 110 and 180 million $m^3$ of timber (Wermelinger, 2002). ‘Kyrill’ in

2007 was one of the most devastating Central European storms of the last decades (Klaus et al., 2011). Those natural disasters provided a large amount of suitable breeding material with the result of extensive *I. typographus* mass propagations and dramatic tree mortality (Schelhaas et al., 2003; Wermelinger, 2004). For example, spruce area in the central part of the Bavarian Forest National Park (Germany) was reduced by more than half from 10700 to 4800 ha between 1990 and 2010 (Kautz et al., 2011). Between 1950 and 2000, approximately 3 million $m^3$ of wood were damaged annually by bark beetles in Europe, mainly by *I. typographus* (Schelhaas et al., 2003). Natural population dynamics without interference from human control measures were rarely observed. However, for instance in the Bavarian Forest or Harz National Parks in Germany, it has been observed that outbreaks decline after a certain time (approximately three to six years, e.g. Heurich et al., 2001; Rall and Martin, 2002; reviewed in Wermelinger, 2004). Recent studies suggest more or less periodic outbreak cycles in the Bavarian Forest National Park (see Fig. 3 in Kautz et al., 2011 and Fig. 5 in Lausch et al., 2013), as it is typical for other forest herbivores, especially some lepidopterans (Dajoz, 2000).

Despite extensive studies of biological characteristics in the last century, population dynamics in *I. typographus* is far from being completely understood or even predictable (Wermelinger, 2004). On the one hand, mass propagations are favoured by natural disturbances on landscape level. On a more local scale, they are influenced by host plant availability and degree of tree susceptibility. Tree susceptibility is modulated by stand parameters such as water and nutrient availability, tree species mixture, exposition or elevation. On the other hand they may be decelerated after a certain time by negative feedback mechanisms such as intraspecific competition or antagonist population dynamics, among others (Schroeder, 2007; Wermelinger, 2002; 2004). Studying conditions of early development and their influence on fitness and behaviour might substantially improve the understanding of population dynamics (Metcalfe and Monaghan, 2001). They have been shown to affect survival, reproduction and offspring quality in numerous bird and mammal species (Lindström, 1999). In insects, varying conditions of larval development may severely influence physiological, morphological or behavioural traits in imagines. Factors such as poor host plant quality or intraspecific competition (i.e. high population densities) lead to reduced size, fecundity, longevity or dispersal capacity across a number of insect taxa (Sallé and Raffa, 2007 and references therein).

Body weight, size and lipid reserves are closely related to those life history traits. They are regularly used as fitness parameters for animals from insects to vertebrates (e.g. Anderbrant

and Schlyter, 1989; Honěk, 1993; May, 1992; Peig and Green, 2009; Savage et al., 2004; Schulte-Hostedde et al., 2005). In most insects lipids in form of triacylglycerols are the main energy reserves (Arrese and Soulages, 2010). The primary site of their production and storage is the diffuse fat body located in the abdomen (Hahn and Denlinger, 2011). These reserves are crucial for detoxification of host plant compounds (e.g. Gatehouse, 2002), survival success of overwintering insects (e.g. Hahn and Denlinger, 2011) or insect flight (e.g. Candy et al., 1997). Therefore, body weight, size and lipid content are used as individual fitness parameters throughout this study. The study will concentrate on the influence of host tree metabolites, population density and overwintering biology on *I. typographus* fitness parameters. Furthermore, the translation of variable lipid reserves into flight capacity will be analysed. Implications for population dynamics, potential effects of expected climate change and consequences for forest management strategies will finally be discussed in a synopsis of the different chapters.

*Host defence and colonisation*

To successfully colonise vigorous trees, a larger number of individuals has to attack more or less simultaneously (Raffa, 2001; Rudinsky, 1962). Primary attraction and host selection are still not fully understood (Hietz et al., 2005). Mid-range visual (e.g. Campbell and Borden, 2009) and short-range gustatory cues (e.g. Raffa and Berryman, 1982b) as well as abiotic factors such as insolation (Kautz et al., 2013) are involved in primary attraction. Insolation most likely enhances the emission of kairomones in form of volatile host metabolites (Hietz et al., 2005), which also play an important role in the primary attraction of *I. typographus* (e.g. Reddemann and Schopf, 1996; Rudinsky et al., 1971; Schroeder and Lindelöw, 1989). It is widely accepted that, after boring into the bark, the pioneer beetles, i.e. the first colonising beetles, commence a much stronger, secondary attraction of conspecifics via aggregation pheromones released with their frass (Blomquist et al., 2010; Gitau et al., 2013; Vité and Francke, 1976, but compare Lehmberg, 2013).

The initial host colonisation period implies high risks for pioneer beetles. The secondary phloem of conifers is rich in organic nutrients and therefore targeted by a variety of species across different phyla, from fungi and bacteria to insects and vertebrates (Franceschi et al., 2005). Thus, conifers have developed several potent constitutive and inducible defences against herbivores. The first lines of defence are non-specific, mechanical and chemical traits aiming to prevent penetration of the bark. The periderm consists of the phellogen (cork cambium) and its outwards product phellem (cork tissue) and inwards product phelloderm. It

controls gas exchange in the stem and at the same time serves as mechanical barrier against intrusion. The phellem consists mainly of dead cell layers with lignified or suberised walls. They can also contain calcium oxalate crystals as an additional mechanical barrier (Franceschi et al., 2005). Furthermore, phellem cells can contain significant amounts of phenols (Franceschi et al., 2005), which are supposed to serve as defence against herbivores and fungi (Beckman, 2000). To allow for gas exchange, the periderm is interrupted by lenticels, which may serve as an entry point for bark beetles (Rosner and Führer, 2002). Main constitutive defence mechanisms in conifers are located in the secondary phloem. It contains sclerenchyma cells, calcium oxalate crystals and polyphenolic parenchyma cells. Whereas lignified sclerenchyma cells and calcium oxalate crystals can serve as mechanical barrier against any kind of herbivore, polyphenolic parenchyma cells contain phenolic bodies in their vacuoles as chemical defence in addition to their thickened cell walls (Hudgins et al., 2004; Hudgins and Franceschi, 2004). In polyphenolic parenchyma cells considerable amounts of starch or lipids are stored which most likely makes them a highly attractive target for herbivores. Thus, in addition to the function as mechanical and chemical barrier against fungal penetration, the constitutive phenols may serve as chemical protection for the cells themselves (Franceschi et al., 2005; Franceschi et al., 2000). Furthermore, most conifer species contain radial and axial resin ducts and resin blisters surrounded by resin cells (Bohlmann, 2008). Resin cells synthesize resins containing terpenoids which are released in the extracellular lumens. When the bark gets wounded by an intruding organism, the sticky resin can trap the invader or even flush it out, because the resin is under pressure in the extracellular lumen (Franceschi et al., 2005; Langenheim, 1994). Moreover, resin terpenoids are toxic for insects and can even be lethal (Langenheim, 1994; Raffa et al., 1985). Efficiency of constitutive resin ducts can be increased by interaction with additionally induced resin ducts (Franceschi et al., 2005; Nagy et al., 2000).

The second levels of defence are inducible structural mechanisms. They can repair damaged tissue and isolate intruding insects. The ‘hypersensitive response’ occurring at attack sites results in reactive oxygen species production and rapid cell death. Thus, fungi or bacteria are contained locally (Bleiker and Uzunovic, 2004). Formation of callus tissue which becomes lignified or suberised in the process is a more general reaction. It also aims to provide a barrier to invading organisms. Wound periderms can also confine wounded regions of bark tissue. Wound periderm is cutting off damaged tissue from nutrient supply and is usually formed around bark beetle induced injuries (Franceschi et al., 2005). Finally, most important against pest species are inducible chemical defences which include non-protein and protein

based chemicals. Protein-based defence compounds comprise toxic proteins such as porins or lectins, enzymes such as chitinases or glucanases and enzyme inhibitors such as proteinases or amylase inhibitors. The inducible enzymes can either promote lignification of tree cell walls or, as in the case of enzyme inhibitors, directly decrease performance of the invading organism, for example by reducing digestion (Franceschi et al., 2005). They can be highly specific as it has been shown for a particular subset of chitinases in *P. abies* which is up-regulated when attacked by the fungus *Heterobasidion annosum* Brefeld (Hietala et al., 2004). The advantage of non-protein compounds like phenolics, terpenoid resins or alkaloids is that they are effective against many organisms and can be produced more rapidly than protein based compounds (Franceschi et al., 2005). It has been found that wounding of bark causes accumulation of phenolic compounds, which possess antifungal and antifeedant properties (Kusumoto and Suzuki, 2003). They can prevent further intrusion into tree tissues by binding hydrolytic enzymes secreted by invading organisms (Appel, 1993; Nicholson and Hammerschmidt, 1992). Furthermore, phenolics reduce the nutrient value of tissues by binding amino acids and proteins and they can even reduce digestion of invaders by binding to digestive gut enzymes (Franceschi et al., 2005). In addition to constitutive resin ducts, additional traumatic resin ducts can be induced in the surroundings of intrusions. As a result, production of terpenoid resins can be extensively enhanced (Krekling et al., 2004; Lombardero et al., 2000b; McKay et al., 2003). The resin of traumatic ducts can be even more toxic than that of constitutive ducts by altering terpene composition or by addition of phenolic metabolites (Nagy et al., 2000). The complexity may hinder bacteria, fungi and herbivores to evolve resistance to all compounds contained in the mixture (Langenheim, 1994).

Through aggregation via the efficient mechanism of secondary attraction, aggressive bark beetle species are believed to reduce this toxicity because the large number of attackers quickly depletes resin reservoirs and furthermore kills the host tree before an additionally induced resin production can commence (Franceschi et al., 2005; Raffa and Berryman, 1987). In some bark beetle species, emission of de-novo synthesised aggregation pheromone components is terminated when the constitutive resin reservoirs are depleted (e.g. Raffa and Berryman, 1983). Moreover, anti-aggregation pheromones are released in some species when certain colonisation densities are reached, i.e. when tree defence is overwhelmed (verbenone and ipsenol in *I. typographus*, Blomquist et al., 2010; Gitau et al., 2013; Pureswaran et al., 2000; Schlyter et al., 1989). It is supposed that the vertical larval feeding activity disrupts nutrient transport and thus kills the tree (Postner, 1974). But usually, associated virulent blue-stain fungi, which are common in bark beetles (Paine et al., 1997), are thought to kill the tree

before girdling activity of beetle larvae does (Christiansen, 1985a; b; Krokene and Solheim, 1996; 1998).

It is striking that serious aggressive bark beetle pests appear only in the Pinaceae, which possess the best developed resin system within conifers (Franceschi et al., 2005). Usual monoterpene concentrations in healthy conifers are toxic to all developmental stages of several scolytid species (e.g. Berryman and Ashraf, 1970; Raffa et al., 1985; Smith, 1963). However, aggressive bark beetles seem to have turned the host defence system partly to their advantage in a plant-herbivore 'co-evolutionary arms race'. Firstly, they are using kairomones in form of volatile host terpenoids for primary attraction (Reddemann and Schopf, 1996; Rudinsky et al., 1971). Secondly, aggregation pheromones participating in secondary attraction most probably evolved from detoxification products of host monoterpenes (Franceschi et al., 2005; Hunt and Smirle, 1988). For example, one of the main components of *I. typographus* aggregation pheromone is cis-verbenol (Vité et al., 1972). It cannot be synthesized de novo by the beetles but rather has to be oxidized from its precursor, the spruce monoterpene (-)-α-pinene (Blomquist et al., 2010). As a result, some scolytids were obviously able to turn the well developed defence mechanisms into a weakness.

Nevertheless, independent of their aggressiveness, bark beetle progeny requires dying, i.e. non-resistant, host tissues for successful development (Raffa et al., 1993). Thus, especially in the early stage of infestations when tree defence is still high, beetles need to consume energy for detoxification of secondary tree metabolites (Gatehouse, 2002; Gries et al., 1990; Raffa et al., 2005). Because they are expensive to produce, terpenoids may be considerably variable between tree individuals depending on their physiological condition, attack density or even within trees (Franceschi et al., 2005; Langenheim, 1994; Raffa and Berryman, 1982a). Furthermore, nutritional value of phloem may be variable between trees and/or reduced with increasing attack density and the resulting intensity of induced reaction. The feeding activity of larvae, which leads to phloem girdling may additionally handicap nutrient allocation to lower tree parts (Postner, 1974). With progressing infestation, the feeding substrate deteriorates more and more, which may lead to less fit progeny from later deposited eggs (Botterweg, 1983). Therefore, the distribution of important nutrients and toxic metabolites within *P. abies* trees was analysed and compared to fitness parameters of beetles developing at different stem heights in the first chapter of this study. The effect of larval feeding on metabolite allocation was studied using artificial tree girdling. Nutrient depletion with advancing infestation progression was simulated by starving of beetles and influence on beetle fitness was analysed.

*Population density and competition*

After successfully overwhelming the defence system of a host tree, colonisation density plays a crucial role for individual fitness and population dynamics. On the one hand, *I. typographus* requires a high attack density for successful colonisation. On the other hand, high colonisation densities may lead to intraspecific competition among parental beetles and their progeny. Therefore, population density and thus intraspecific competition may have considerable effects on population dynamics and the regulation of *I. typographus* populations (Anderbrant and Schlyter, 1989; Okland and Berryman, 2004). Comparative studies considering conditions of larval development might help considerably in explaining these effects.

Intraspecific competition can occur in two characteristics, interference and exploitation competition. In case of interference competition individuals interact directly, for instance when one individual interferes with reproduction or the establishment within the habitat of another. Exploitation competition occurs without direct contact of individuals when common resources are limited. Fitness of an individual is decreased when the availability of a resource has been depressed by another (Begon et al., 2006). In high population densities, larvae as well as imagines are confronted with a mix of both forms of competition. Whereas among larvae exploitation competition predominates (i.e. competition for food and space), it is the other way round among imagines. Apart from the obvious interference when searching for a suitable site to enter the bark or competing for mating partners, it has been shown that parental beetles leave their hosts sooner when larger numbers of conspecifics are present in trees (Anderbrant, 1985; 1986; Anderbrant et al., 1985; McMullen and Atkins, 1961). As a result, length of maternal galleries and oviposition per female are reduced in numerous bark beetle species (Anderbrant, 1990; Aukema and Raffa, 2002; Beaver, 1974; Coulson, 1979; Eidmann and Nuorteva, 1968; Light et al., 1983; McMullen and Atkins, 1961; Wagner et al., 1981; Weslien, 1994). Parental beetles forced to leave sooner due to high colonisation density disperse to seek another tree for a second brood (Anderbrant, 1989). This might reduce population growth even further, either by dispersal losses or reduced overall oviposition per female, as additional energy is consumed during the second dispersal which then may be lacking for detoxification of host tree defence metabolites and reproduction. Offspring production in *I. typographus* significantly decreases with increasing population density, in wind-felled as well as in standing trees (Komonen et al., 2011). Moreover, offspring mortality rates are higher in high population densities (Anderbrant, 1990; Beaver, 1974; Eidmann and Nuorteva, 1968). To some extent, even cannibalism may occur among larvae developing in high densities (de Jong and Grijpma, 1986).

Since females only partly compensate for high attack density by decreasing egg number (Anderbrant and Schlyter, 1989), exploitation competition plays a major role in determining fitness of surviving offspring and thus for modulation of population growth. Numerous studies demonstrated a negative influence of population density on scolytid progeny fitness, in terms of decreased weight, size, fecundity or lipid content (e.g. Amezaga and Garbisu, 2000; Anderbrant et al., 1985; Atkins, 1975; Beaver, 1974; Botterweg, 1983; Sallé et al., 2005). Several authors have reasoned that low lipid reserves translate in reduced flight capacity (e.g. Anderbrant, 1985; Gries, 1985; Williams and Robertson, 2008), whereas other authors reported that flight capacity is independent of size or lipid content (e.g. Botterweg, 1982; Forsse and Solbreck, 1985). Therefore, fitness parameters in terms of body weight, size, lipid reserves and dispersal capacity were studied in the second chapter, comparing beetles which have developed in different population densities.

*Overwintering biology and diapause*

Overwintering behaviour including diapause is one of the few aspects of basic *I. typographus* biology which is not yet understood very well (Wermelinger, 2004). Diapause is a life cycle strategy which enables individuals to bridge conditions that are unfavourable for development and reproduction, which for instance include hot summer or cold winter months. Hence, it synchronises active stages with favourable conditions (Danks, 1987; Tauber et al., 1986). Additionally, in bark beetle species which require simultaneous mass attack for successful host colonisation, diapause may contribute to synchronisation of dispersal onset within a population in the spring swarming period. A characteristic feature of diapause is that it is genetically programmed. In species with a facultative diapause its onset is triggered by environmental conditions experienced by individuals themselves or their parents, whereas in species with an obligate diapause all individuals commence it at the same point in their life cycle in every generation (Denlinger, 2002; Tauber et al., 1986). Generally, diapause is characterised by developmental arrest and metabolic depression with a decreased intermediate and respiratory metabolism (Guppy and Withers, 1999; Hahn and Denlinger, 2007). Metabolic depression can also include functional incapacitation or degeneration of highly energy consuming tissues such as the flight muscles (e.g. de Kort, 1990; Doležal and Sehnal, 2007a; b). However, diapause is a graded, dynamic process of distinct physiological stages with temporal patterns of gas exchange, nutrient metabolism, stress resistance and gene expression. It does not necessarily require a complete standstill of development (Koštál, 2006). Physiological changes include down-regulation (e.g. cell growth and proliferation),

up-regulation (e.g. heat shock proteins or cryoprotectants) or reduced levels of metabolic pathways (e.g. basic cellular maintenance) (Storey and Storey, 2004; 2012). Also, most insect species have in common that they do not, or only at a minimum, feed during diapause which saves energy costs of digestion (Storey and Storey, 2012; Tauber et al., 1986). Thus individuals face the challenge of storing sufficient energy reserves to maintain basic metabolism and to resume activity such as rebuilding atrophied tissues, dispersing or reproducing after diapause termination (Hahn and Denlinger, 2007). When diapausing during winter, considerable amounts of energy reserves are required for the production of cryoprotectants such as glycerol (Denlinger, 2002; discussed in detail in chapter 3). Lipids in form of triacylgylycerols are by far the most important energy reserves used during diapause in insects (Hahn and Denlinger, 2011). In cold hardy insects the proportion of unsaturated fatty acids increases during diapause (Joanisse and Storey, 1996) and in freeze tolerant species membrane phospholipid composition is additionally altered with a shift to more unsaturated fatty acids (Overgaard et al., 2009).

From an evolutionary point of view, it is important to relate diapause occurrence and duration to other life cycle traits which may compensate these costs (Hahn and Denlinger, 2007; Soula and Menu, 2003). As outlined above, the benefit of diapause is obviously a higher survival probability under adverse environmental conditions. On the other hand, diapausing is usually paid by reduced fecundity (due to a reduced reproductive period) and decreased post-diapause fitness (due to the depletion of energy reserves). It has been frequently shown that lipid reserve decline increases with diapause duration (Danks, 1987; Tauber et al., 1986). In *I. typographus* individuals can lose more than 50% of their reserves (e.g. Botterweg, 1982; Kraußе-Opatz et al., 1995). Depending on the developmental overwintering stage and the location of overwintering (within the host tree vs. in the soil litter), winter mortality can decrease *I. typographus* populations up to 50% (e.g. Faccoli, 2002), in extreme cases even up to 100% (Annila, 1969).

Overwintering success and post-diapause fitness, in terms of lipid reserves and dispersal capacity, were tested along a temperature cline in the third chapter of this study. Furthermore, since studies of overwintering site (soil vs. tree) report mixed results between the northern and central distribution area of *I. typographus*, and in some cases even within its central distribution across different years (e.g. Annila, 1969; Biermann, 1977; Botterweg, 1982; Kuhn, 1949; Zumr, 1982), an underlying mechanism which may explain soil overwintering was investigated. Accumulation of energy reserves for diapause has to be initiated well before the start of unfavourable conditions (Denlinger, 2002; Hahn and Denlinger, 2007; Koštál,

2006), which can mean missing the chance of an additional reproductive cycle. Therefore, in the final part of the chapter, time of hibernation onset within a season was studied over a period of three years in a field population in the Bavarian Forest National Park (Germany).

*Flight capacity and dispersal*

Dispersal flight is a short phase of the bark beetle life cycle which occurs mainly between generations, either after overwintering or, in multivoltine populations, to initiate a summer generation. Moreover, in several bark beetle species it regularly occurs for the initiation of sister broods after the first oviposition (e.g. Anderbrant, 1989; Anderbrant and Löfqvist, 1988; Flamm et al., 1987; Sauvard, 1993; Wagner et al., 1981). Dispersal plays a major role in the infestation process, since the host tree usually dies during the development of one generation of beetles (Christiansen, 1985b; Postner, 1974). At least in endemic population phases, suitable host trees represent ephemeral resources which are scattered within the habitat. Thus, dispersing to locate new suitable host trees is obligatory for the progeny of *I. typographus*. Despite the extensive amount of research on bark beetle biology, there is a lack of systematic knowledge about dispersal capacities of *I. typographus* and bark beetles in general (Preisler et al., 2012). To understand dispersal, knowledge about factors influencing flight capacity on individual level as well as flight capacity distribution on population level is essential. In the past, it has been hypothesised that the amount of lipid reserves, which is determined by conditions of development, modulates *I. typographus* flight capacity (see above). A general problem of proving this hypothesis are the destructive methods required for lipid content analysis in insects which imply killing them (Williams and Robertson, 2008). These methods make it impossible to study fluctuations of lipid reserves in the same individual over time. Using comparisons to reference individuals, it has been shown that lipids play a major role in fuelling bark beetle flight (e.g. Williams and Robertson, 2008). However, until now, flight capacity was never directly linked to lipid content within the same individuals. Therefore, in the fourth chapter, the relationship between lipid reserves and flight capacity was studied on individual level using nuclear magnetic resonance microscopy as a novel tool in entomology. This non-invasive method allows analysing water and lipid contents of one individual several times, with the possibility to conduct behavioural assays in between measurements. Most recently, NMR microscopy has been used to visualize internal morphology of several arthropod species (e.g. Gassner and Lohman, 1987; Goodman et al., 1995; Haddad et al., 2004; Tomanek et al., 1996; Wecker et al., 2002). However, the better part of these studies analysed dead specimens, and dynamic physiological traits could not yet be linked directly to

behavioural ones. Finally, in the last chapter, distributions of flight capacities on population level are compared between several laboratory generations with different developmental background and a field population from the Bavarian Forest National Park. As yet, such data are scarce and will possibly improve the development of reliable simulation models of bark beetle dispersal (Preisler et al., 2012).

# Chapter 1: Influence of host tree metabolites on beetle fitness

## 1.1 Introduction

The colonization process within a tree usually starts below the crown and advances downwards the stem (Benz and Zuber, 1990; Jahn, 1990; Niemeyer, 1978). On the one hand this might be rooted in a differing vertical distribution of tree metabolites. On the other hand, the phloem girdling larval feeding activity might reduce nutrient transport in the phloem (Postner, 1974). Thus, larvae developing in the lower parts of the stem would suffer the disadvantage of having to feed more substrate for the same energy uptake. Physiological state of the brood material may have a considerable effect on *I. typographus* offspring. Beetles developing in standing trees are larger than beetles developing in cut logs (Botterweg, 1982). Since cut logs usually dry out fast and no nutrient transport takes place, this suggests that the amount of nutritional content influences offspring fitness, because beetles may only partly be able to compensate low nutritive value by enhanced feeding. Furthermore, reproductive rate in terms of emerging offspring is higher in tree-tops than in lower stem parts (Komonen et al., 2011). On the other hand, the ability of the host tree to produce secondary defence metabolites may increase parental and larval mortality, and may also reduce offspring fitness. Phenolics and terpenoids are the major compounds of chemical defence in conifers. Parenchyma cells, which are specialised to synthesise phenols in their vacuoles, are the most abundant living cell type in the secondary phloem of conifers. Thus, they may be regarded as the most important cell type in conifer defence (Krokene et al., 2008). Phenolics can reduce nutritive value of tree tissues and also reduce digestive activity of insects (Franceschi et al., 2005). Terpenoids represent mechanical defences in form of sticky or crystallized resins, and moreover can be toxic for insects and associated pathogens. They can interrupt essential biological processes in insects, for example by interfering with their endocrine system (Berryman and Ashraf, 1970; Bohlmann, 2008; Raffa et al., 1985; Smith, 1963). Thus, terpenoids may also increase mortality and reduce offspring fitness. However, it is more complicated because in the co-evolution between *P. abies* and *I. typographus* beetles evolved to use volatile host monoterpenes as kairomones to locate suitable host trees (Franceschi et al., 2005; Reddemann and Schopf, 1996; Rudinsky et al., 1971). In the case of the major aggregation pheromone compound cis-verbenol, they even rely on the spruce monoterpene precursor (-)-α-pinene (Blomquist et al., 2010). This usage of host compounds as pheromone precursors is thought to have developed from a detoxification process, because oxidation of monoterpenes enhances

water solubility and thereby excretion rate (Hunt and Smirle, 1988; Reddemann and Schopf, 1996). This process requires energy because NADPH serves as substrate for this enzymatic process (White et al., 1979). Thus, beetles have to feed on toxic substrate and are constrained to expend energy reserves for detoxification of host tree compounds (Gatehouse, 2002; Gries et al., 1990; Raffa et al., 2005; Reddemann and Schopf, 1996). Therefore, they should favour tissues with high nutrient content for egg deposition to ensure high offspring fitness, but on the other hand tissues with low defence metabolite concentration to minimise their own mortality and energy expenditure and that of their offspring, respectively.

In this chapter, vertical distribution of nutrients and toxic metabolites within stems of non-infested *P. abies* tress was analysed. Furthermore, the effect of interrupting nutrient allocation through larval feeding was studied by artificial girdling of spruces. Additionally, fitness parameters of beetles emerging from different stem heights of infested trees were compared. To simulate a more controlled differing nutritional state of beetles, beetles were starved and the effect on fitness parameters was tested.

## 1.2 Methods

### 1.2.1 Tree metabolite contents at different stem heights of non-infested spruces

*Experimental design*

On May 15$^{th}$ 2007, at the end of *I. typographus* spring dispersal, six non infested spruces were selected on the plot 'Grandl' in the National Park Bavarian Forest, Germany (49°5'6.37" N 13°12'46.73" E, 750 m a.s.l.). With the help of tree climbers, four phloem samples of 2 cm in diameter were taken at each 1.5, 5 and 10 m stem height from each tree. Samples were immediately frozen in liquid nitrogen in the field and then stored at -80°C until analysis. The sampling was repeated on May 30$^{th}$ and June 18$^{th}$. The time period between mid May and mid June in large parts covered the time of larval development and maturation feeding. Concentrations of starch, protein amino acids, the soluble carbohydrates glucose, fructose and sucrose, total phenolic compounds and concentrations of the monoterpenes (-)-α-pinene, (+)-α-pinene, (-)-β-pinene, (+)-β-pinene, (-)-limonene, (+)-limonene, (-)-camphene and myrcene of each sample were analysed.

*Analysis of tree metabolites*

The frozen phloem samples were homogenised in a swing mill containing liquid nitrogen with a frequency of 50 Hz. Afterwards, homogenised samples were lyophilised for 48 h in a vacuum atmosphere with an atmospheric pressure of 0.6 bar and a temperature of -76°C. 50 mg of the homogenised, freeze-dried samples were extracted with 500 µl of a 50% v/v methanol/water solution for 20 min in an ultrasonic bath filled with ice-water. Extracts were centrifuged at 4000 rpm and pellets were extracted again three times. Supernatants were used for analysis of phenols and soluble carbohydrates, and dried pellets for protein amino acid analysis.

The soluble carbohydrates glucose, fructose and sucrose were analysed after Schopf (1986). Carbohydrates were converted into trimethylsilyls using an oximation reagent consisting of a 2.5% hydroxylammonium chloride solution (Carl Roth GmbH, Karlsruhe, Germany). Afterwards 1 µl of each sample was analysed using a Varian Saturn 2200 GC-MS system (Agilent Technologies, Santa Clara, CA, USA). The system was equipped with a CP-Sil-8CB capillary column (30 m*0.25 mm i.d. *0.12 µm, Agilent Technologies, Santa Clara, CA, USA). Helium was used as carrier gas. Oven temperature started at 50°C and was increased to 110°C in steps of 10°C $min^{-1}$, followed by an increase to 163°C in steps of 3°C $min^{-1}$, then to 226°C in steps of 7°C $min^{-1}$, afterwards to 265°C in steps of 2.5°C $min^{-1}$ and finally to the end temperature of 300°C in steps of 10°C $min^{-1}$, which was held for 5 min. All components were identified and quantified by comparison of retention times and spectra of the respective synthetic substances. Phenyl-α-D-glucopyranoside (Sigma-Aldrich, St. Louis, MS, USA) was used as internal standard.

Concentration of total phenolic compounds was analysed after Swain and Hillis (1959). The analysis is based on the reducing nature which all phenolic compounds have in common. Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), a mixture of phosphomolybdate and phosphotungstate, was used as oxidant agent. When reduced, the colour of the reagent changes from yellow to blue. Absorbance of the blue complex was measured in a photometer (Perkin-Elmer Lambda2, Waltham, MA, USA) at 750 nm and compared to a catechin (Sigma-Aldrich, St. Louis, MO, USA) standard curve.

Proteins from extraction pellets were hydrolysed with 6 M hydrochloric acid to cleave them into amino acids. Then amino acids were converted into a purple chelat complex with ninhydrin. Absorbance of the complex was measured in a photometer (Perkin-Elmer Lambda2, Waltham, MA, USA) at 570 nm and compared to a norleucine (Sigma-Aldrich, St. Louis, MS, USA) standard curve.

For analysis of starch concentration, starch in freeze-dried phloem samples was cleaved into glucose units with amyloglucosidase. Addition of hexokinase converted glucose in glucose-6-phosphate (G-6-P). Addition of G-6-P-dehydrogenase converted G-6-P to 6-phospho-gluconate. Thereby, one $NADP^+$ molecule is reduced to NADPH which was measured in a photometer (Perkin-Elmer Lambda2, Waltham, MA, USA) at 340 nm and compared to a glucose standard curve. Afterwards, concentrations of free glucose and sucrose obtained by GC-MS analysis (see above) were subtracted to obtain the concentration of glucose originating from starch only.

Concentrations of the monoterpenes (-)-α-pinene, (+)-α-pinene, (-)-β-pinene, (+)-β-pinene, (-)-limonene, (+)-limonene, (-)-camphene and myrcene were measured using GC-MS analysis. Therefore, two small pieces were cut from each phloem sample and extracted in 200 µl hexane (Carl Roth GmbH, Karlsruhe, Germany) for 24 h. Afterwards, 1 µl of each phloem sample extract was analysed on a Varian Saturn 2200 GC-MS (Agilent Technologies, Santa Clara, CA, USA). The GC-MS system was equipped with a WCOT fused silica CP-Cyclodextrin-β-2.3.6-M-19 capillary column (50 m*0.25 mm i.d. *0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The mass range was 40 to 200. Helium was used as carrier gas. The oven temperature started at 40°C for 5 min, followed by an increase of temperature with steps of 5°C $min^{-1}$ to 80°C held for 10 min, again followed by an increase of temperature with steps of 3°C $min^{-1}$ to 160°C and then with steps of 20°C $min^{-1}$ to 220°C. All components were identified and quantified by comparison of retention times and spectra of the respective synthetic substances. Nonan was used as internal standard. After GC-MS analysis, samples were dried at 60°C for 24 h and their dry weight was measured.

*Statistical analysis*

To account for possible seasonal variation in the analysed metabolites, mean concentrations per dry weight of the single metabolites were calculated per stem height and tree across all three sampling dates. Afterwards, concentrations of the different compounds were compared between stem heights using an Approximative General Independence Test for complete block designs with 10,000 Monte Carlo replications, followed by a Wilcoxon-Nemenyi-McDonald-Thompson (WNDT) post-hoc test (for details see Hollander and Wolfe, 1999), accounting for dependant samples within each tree. Therefore, the ‘symmetry_test’ function within the package ‘coin’ (Hothorn et al., 2009) of the R2.14.1-system (R Development Core Team, 2011) was used.

### 1.2.2 Tree metabolite contents above and below artificial phloem girdling

*Experimental design*

On May 15th 2007, twelve non infested spruces were selected on each of the plots 'Grandl' (49°5'6.37" N 13°12'46.73" E, 750 m a.s.l.) and 'Geissberg' (48°55'47.08" N 13°20'39.01" E, 800 m a.s.l.) in the National Park Bavarian Forest, Germany. On each plot, six trees were girdled at 1.5 m stem height, i.e. a 3 cm wide strip of bark was removed completely around the stem. The other six trees per plot remained untreated. On June 18th 2007, four phloem samples of 2 cm in diameter were taken 10 cm below and 10 cm above the artificial girdling. From each untreated tree four phloem samples were taken at 1.5 m stem height. Samples were immediately frozen in liquid nitrogen in the field and stored at -80°C until analysis. Concentrations of starch, protein amino acids, glucose, fructose, sucrose, total phenolic compounds and total concentration of the sum of monoterpenes of each sample were analysed (see chapter 1.2.1 for detailed methods).

*Statistical analysis*

Concentrations of analysed tree compounds were compared within each tree between below and above the artificial girdling using a paired-sample Wilcoxon signed rank test. Concentrations of compounds below the artificial girdling were compared to concentrations of untreated trees at the same stem height with a Mann-Whitney U-test. All statistical analyses were conducted using R version 2.14.1 (R Development Core Team, 2011).

### 1.2.3 Fitness parameters of beetles developing at different stem heights

*Experimental design*

In 2008 and 2009, emerging first generation beetles in the Bavarian Forest National Park were caught to analyse fitness parameters. Therefore, passive traps were installed at six infested trees on the plot 'Grandl' (49°5'6.37" N 13° 12'46.73" E) and at six trees on the plot 'Geissberg' (48°55'47.08" N 13°20'39.01" E, 800 m a.s.l.) in June 2008. At each tree three eclector traps for catching emerging beetles were set up, at approximately 1.5 m, 5 m and 10 m stem height. Eclectors consisted of a plastic half-tube with a plastic funnel at its bottom end. The funnel led into a container filled with water to trap beetles. To reduce surface tension, few drops of detergent were added to the water. The opening of the half-tube was directed towards the stem. Thus, emerging beetles bumped against the plastic and were led

through the funnel into the container. Eclectors were sealed with a plastic cover on top (Fig. 1.1). Emerged beetles were collected weekly between July 18th, 2008 and October 8th, 2008 and April 8th, 2009 and June 3rd, 2009, respectively. Sizes, as product of pronotum width and elytra length, of a total of 3405 emerged filial beetles were calculated. Width and length were measured using a stereo-microscope (Stemi SV 11, Zeiss, Oberkochen, Germany) and an ocular with integrated millimetre scale (Zeiss, Oberkochen, Germany). After drying at 60°C for 24 h, dry weight of a total 3373 beetles was measured using an electronic micro scale (Sartorius M5P, Sartorius AG, Göttingen, Germany).

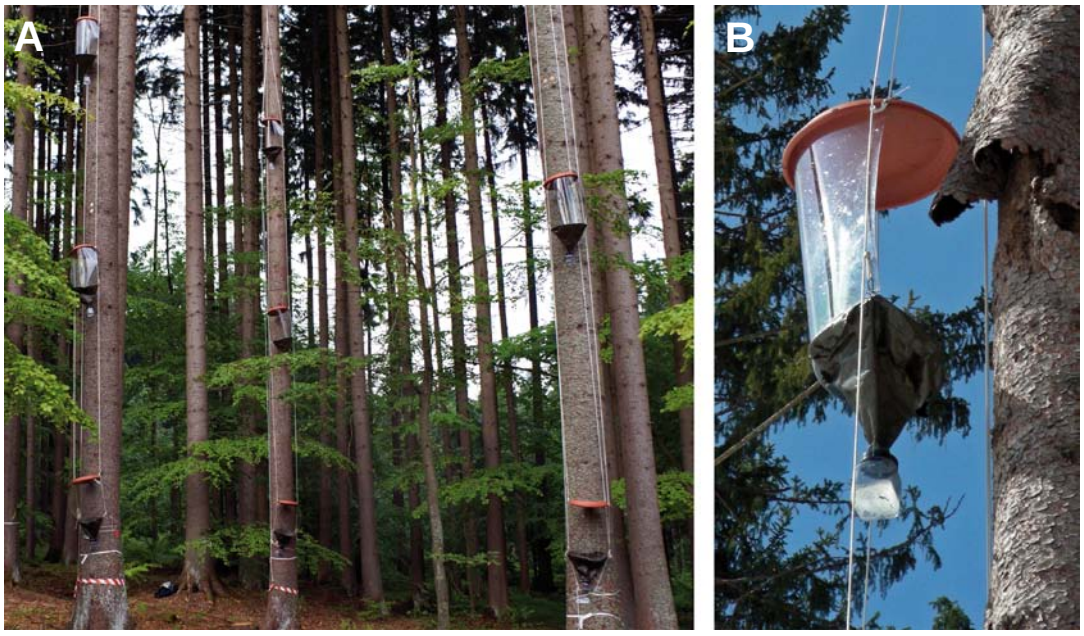


**Fig. 1.1.** Stem eclectors for trapping emerging *I. typographus* at infested trees: **A.** Complete setup and **B.** close-up view of an eclector.

*Statistical analysis*

Mean dry weights and sizes were calculated per stem height and tree across all collection dates and both plots. Afterwards, they were compared between stem heights using an Approximative General Independence Test for complete block designs with 10,000 Monte Carlo replications, followed by a Wilcoxon-Nemenyi-McDonald-Thompson (WNDT) post-hoc test (for details see Hollander and Wolfe, 1999), accounting for dependant samples within each tree. Therefore, the 'symmetry_test' function within the package 'coin' (Hothorn et al., 2009) of the R2.14.1-system (R Development Core Team, 2011) was used.

### 1.2.4 Fitness parameters of beetles differing in nutritional state simulated by starvation

*Experimental design*

Beetles originated from the 34$^{th}$ generation of the laboratory stock of the Institute for Animal Ecology (Technische Universität München, Freising). The stock had been initiated in late 2006 with beetles collected in 'Kranzberger Forst' near Freising, Germany (48°24'30.08" N 11°39'26.63" E, 500 m a.s.l.). It has been maintained on spruce logs of approximately 60 cm length and 20 to 35 cm diameter according to Bohlander (1996). Light conditions were 16:8 (L:D) and temperature was between 20 and 25°C. A collective of newly emerged *I. typographus* individuals was divided into five groups of 30 beetles each. The first group was analysed immediately after emergence from brood logs, the remaining four groups were held at 15°C without nutrition between one and four days. Each group was again divided into sub-groups of 10 beetles of which flight capacity within 24 h was tested in automated flight mills and 20 beetles of which lipid content, fresh and dry mass were analysed. Fresh masses were measured using an electronic scale (Sartorius Basic, Sartorius AG, Goettingen, Germany) and dry masses as described above (chapter 1.2.3).

*Lipid measurement*

For lipid measurement beetles were killed by freezing at -20°C. Afterwards they were cut in three pieces between head and thorax and thorax and abdomen. Fatty acid esters were extracted from the dissected beetles three times in 1 ml chloroform at 30°C in an ultrasonic bath. The chloroform samples were dried at 65°C for 24 h. Lipid reserves were measured photometrically as acylester equivalents according to Snyder and Stephens (1959) and modified after Kraußе-Opatz et al. (1995). The measurement is based on a hydroxylaminolysis, in which an ester group forms a hydroxamic acid when reacting with alkaline hydroxylamine. In the presence of ferric perchlorate the hydroxamic acid forms a purple iron-chelate complex which was measured in a photometer (Perkin-Elmer Lambda2, Waltham, MA, USA) at 530 nm. In contrast to Snyder and Stephens (1959) the final concentration of perchloric acid was ten times higher. Thus, the range of the standard curve's linearity is extended (Bohlander, 1996). For each batch of beetles analysed photometrically, a standard curve for fatty acid esters was generated. It was determined based on ten samples of methyl oleate (Sigma-Aldrich, St. Louis, MS, USA) dissolved in chloroform in concentrations between 0.5 and 5.0 µmol in 0.5 µmol steps (Pearson's product-moment correlation: all $R^2 > 0.98$, all $t > 28$, all $df = 8$, all $p < 0.0001$, all $N = 10$).

*Flight mills*

Flight capacity was tested using automated flight mills. Each of them consisted of a vertical glass microcapillary which served as pivot. To minimize friction between the pivot and the frame of the flight mill, tips of insect needles were attached to each end of the capillary. To reduce weight, a gas chromatography capillary column, perpendicularly attached to the pivot, served as vertical rotary arm. The radius of the rotary arm was 10.5 cm. Hence, one rotation of the arm corresponded to a flight distance of 0.66 m. Beetles were fixated to the rotary arm with a silver wire, which was attached to a beetle's pronotum with a droplet of odour-free super glue (Uhu, Bühl, Germany). Attachment angle was kept consistent among beetles to ensure reproducibility. On the other end of the arm was a counter weight of approximately the beetles' weight (12 mg). At the lower end of the pivot an aluminium disc was attached. It was 2 cm in diameter and had a 2 mm wide notch which was running through a photo sensor attached to the mounting of the pivot (Fig. 1.2). Thus, each rotation triggered the photo sensor and indicated a flight distance of 0.66 m. The signal was transmitted to a computer and recorded on-line in 10 s intervals using DIAdem version 10.0 (National Instruments, Austin, TX, USA, 2005). Only intervals with more than two rotations per 10 s were counted as actual flight. Thus, slight movements of the pivot caused by leg or abdomen movements and minor ventilation respectively, were excluded.

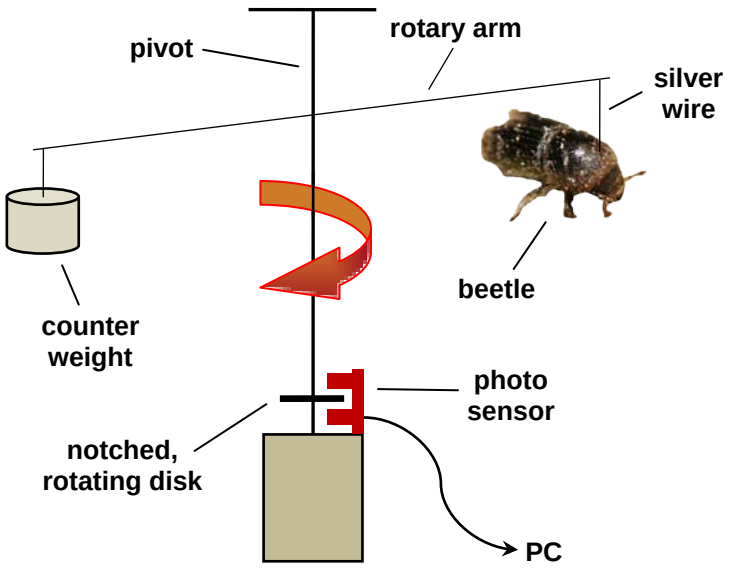


**Fig. 1.2.** Flight mill setup used for testing *I. typographus* flight capacity.

*Statistical analysis*

Fresh and dry masses, lipid content and flight distances were compared between the different periods of starvation using an Approximative K-Sample Permutation test with 10,000 Monte-Carlo replications, followed by a Nemenyi-Damico-Wolfe-Dunn (NDWD) post-hoc test (for details see Hollander and Wolfe, 1999). Therefore, the 'oneway_test' function within the package 'coin' (Hothorn et al., 2009) of the R2.14.1-system (R Development Core Team, 2011) was used.

## 1.3 Results

### 1.3.1 Tree metabolite contents at different stem heights of non-infested spruces

Fructose and protein amino acids were evenly distributed within trees (Approximative General Independence Test, WNDT post-hoc test: all maxT < 2.3, all p > 0.07, all N = 6 trees with 3 samples each), although fructose showed a strong trend for higher concentrations at the upper parts of the stem. All other analysed metabolites were unevenly distributed. Starch, glucose and sucrose were significantly less concentrated at the lower part of the stem (all maxT > 2.60, all p < 0.05, all N = 6), whereas toxic phenols and monoterpenes were significantly higher concentrated at the lower stem segment (all maxT > 3.46, all p < 0.001, all N = 6) (Tab. 1.1 and Fig. 1.3). α- and β-pinene represented more than 90% of analysed monoterpenes (Fig. 1.4).

**Tab. 1.1.** Concentrations of tree metabolites at the three sampled stem heights. Shown are means with standard deviation per height in nmol $mg^{-1}$ dry mass. Differing letters indicate significant differences between heights within each compound (Approximative General Independence Test, WNDT post-hoc test, for pairwise differences see Fig. 1.3).

| Compound | 1.5 m | 5 m | 10 m |
|---|---|---|---|
| Starch | 683.08 ± 74.05$^{a}$ | 719.11 ± 98.81$^{ab}$ | 802.18 ± 111.19$^{b}$ |
| Glucose | 15.66 ± 3.76$^{a}$ | 23.89 ± 9.59$^{b}$ | 24.53 ± 9.82$^{b}$ |
| Fructose | 27.24 ± 12.91$^{a}$ | 31.28 ± 11.49$^{a}$ | 32.54 ± 14.30$^{a}$ |
| Sucrose | 188.91 ± 18.00$^{a}$ | 218.07 ± 18.10$^{b}$ | 220.69 ± 20.96$^{ab}$ |
| Amino acids | 160.75 ± 15.84$^{a}$ | 157.63 ± 9.23$^{a}$ | 150.43 ± 13.32$^{a}$ |
| Phenols | 456.92 ± 22.72$^{a}$ | 403.36 ± 19.89$^{ab}$ | 339.61  21.94$^{b}$ |
| Monoterpenes | 13.84 ± 6.87$^{a}$ | 5.11 ± 2.86$^{ab}$ | 2.02 ± 1.35$^{b}$ |

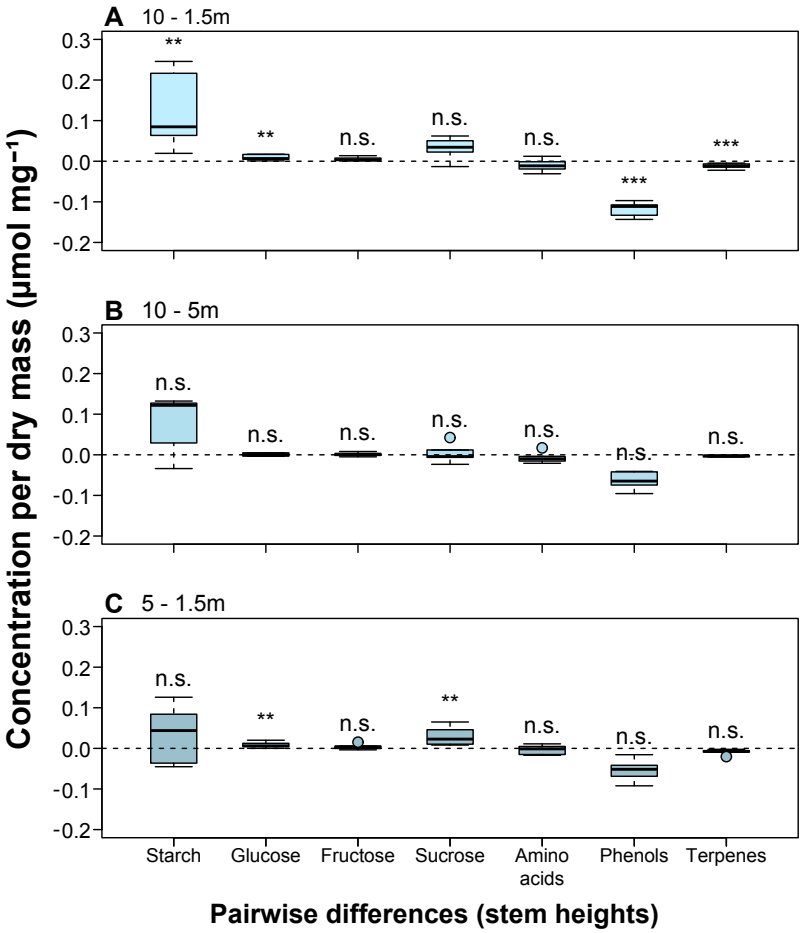


**Fig. 1.3.** Pairwise differences of tree metabolite concentrations between the three sampled stem heights. Difference between **A.** 10 and 1.5 m, **B.** 10 and 5 m and **C.** 5 and 1.5 m. Significance levels are given as *** $p < 0.001$, ** $p < 0.01$ and n.s. = not significant (Approximative General Independence Test, WNDT post-hoc test, for absolute mean values per stem height see Tab. 1.1). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

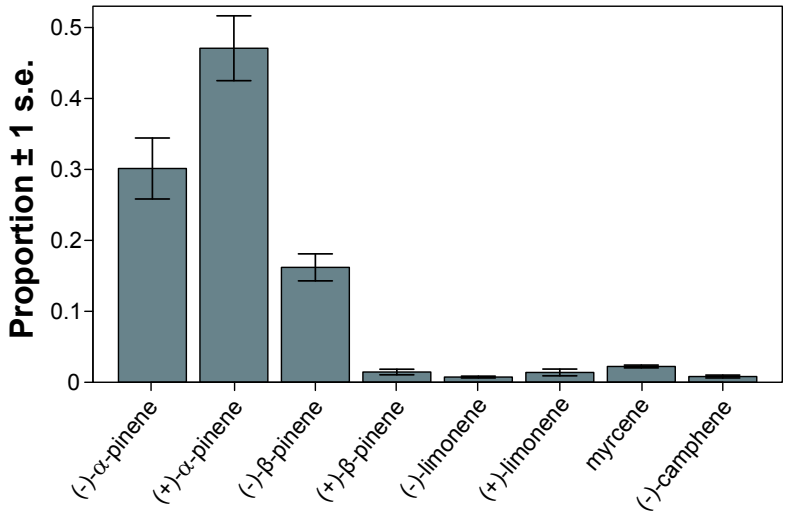


**Fig. 1.4.** Monoterpene concentrations per tree. Shown are means with one standard error (s.e.), N = 6 trees with 9 samples per tree (three sampling times with three stem heights each).

### 1.3.2 Tree metabolite contents above and below artificial phloem girdling

Girdling had a significant impact on assimilate transport. Starch, sucrose and protein amino acids were significantly higher concentrated above the artificial girdling than below it (paired-sample Wilcoxon signed rank test: all $Z > 2.59$, all $p < 0.01$, all $N = 12$ trees with 2 samples each). In contrast, glucose and fructose concentrations were significantly lower above the artificial girdling (all $Z < -2.82$, all $p < 0.01$, all $N = 12$), whereby their total concentrations was one order of magnitude smaller than those of starch, sucrose and protein amino acids. Concentrations of phenols and monoterpenes did not differ between above and below girdling (all $Z > -1.80$, all $p > 0.07$, all $N = 12$ trees with 2 samples each), although both showed a considerable trend for higher concentrations below girdling (Tab. 1.2 and Fig. 1.5).

The nutrients starch, glucose, fructose and sucrose below the artificial girdling were significantly less concentrated than in untreated spruces at the same stem height (Mann-Whitney U-test: all $U < -3.06$, all $p < 0.01$, all $N = 24$ trees with 12 girdled and 12 untreated trees). Amino acids and toxic phenols and monoterpenes showed no difference in their concentrations between girdled and untreated trees (all $U < 1.04$, all $p > 0.30$, all $N = 24$) (Tab. 1.3).

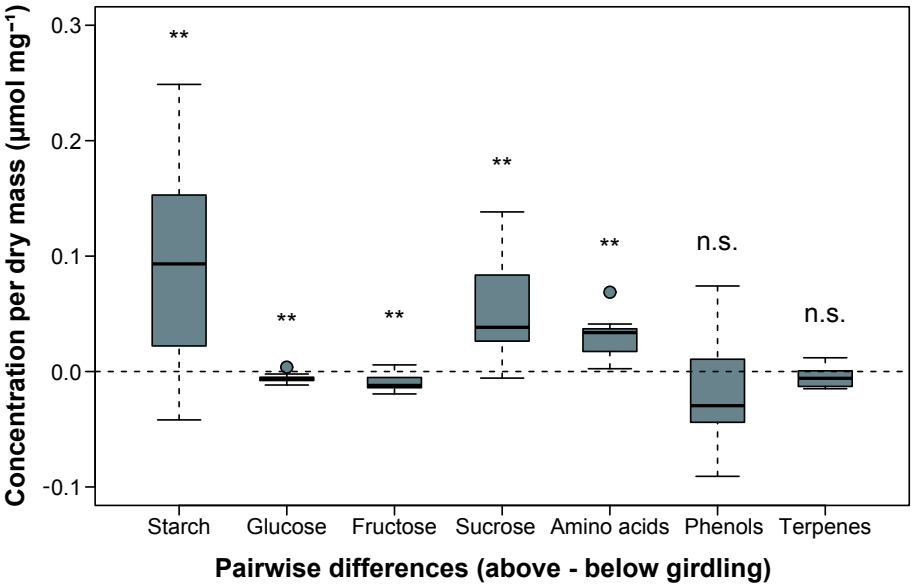


**Fig. 1.5.** Pairwise differences of tree metabolite concentrations between above and below artificial girdling. Significance levels are given as ** $p < 0.01$ and n.s. = not significant (paired-sample Wilcoxon signed rank test, for absolute mean values above and below girdling see Tab. 1.2). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

**Tab. 1.2.** Tree metabolite contents above and below artificial tree girdling. Shown are means with standard deviation per sampling position in nmol $mg^{-1}$ dry mass. Differing letters indicate significant differences between positions within each compound (paired-sample Wilcoxon signed rank test, for pairwise differences see Fig. 1.5).

| Compound | Below girdling | Above girdling |
|---|---|---|
| Starch | 268.59 ± 86.07[a] | 362.39 ± 157.67[b] |
| Glucose | 9.98 ± 3.05[a] | 4.10 ± 1.53[b] |
| Fructose | 15.76 ± 5.45[a] | 5. 75 ± 3.09[b] |
| Sucrose | 168.47 ± 22.85[a] | 221.64 ± 39.38[b] |
| Amino acids | 165.48 ± 14.43[a] | 194.77 ± 22.29[b] |
| Phenols | 405.71 ± 84.73[a] | 389.00 ± 73.88[a] |
| Monoterpenes | 14.38 ± 5.84[a] | 9.58 ± 10.19[a] |

**Tab. 1.3.** Tree metabolite contents below artificial girdling and at the same height of untreated spruces. Shown are means with standard deviation in nmol $mg^{-1}$ dry mass. Differing letters indicate significant differences within each compound (Mann-Whitney U-test).

| Compound | Girdled | Untreated |
|---|---|---|
| Starch | 268.59 ± 86.07[a] | 558.70 ± 115.96[b] |
| Glucose | 9.98 ± 3.05[a] | 17.13 ± 4.99[b] |
| Fructose | 15.76 ± 5.45[a] | 23.16 ± 9.40[b] |
| Sucrose | 168.47 ± 22.85[a] | 206.91 ± 25.31[b] |
| Amino acids | 165.48 ± 14.43[a] | 158.74 ± 15.73[a] |
| Phenols | 405.71 ± 84.72[a] | 407.01 ± 61.65[a] |
| Monoterpenes | 14.38 ± 5.84[a] | 15.22 ± 13.86[a] |

### 1.3.3 Fitness parameters of beetles developing at different stem heights

Dry masses of beetles developing at the lower part of the stem were significantly lower than those of beetles emerging from the upper parts at 5 and 10 m (Approximative General Independence Test, WNDT post-hoc test: maxT = 3.14, $p < 0.01$, N = 12 trees with 3373 beetles in total, Fig. 1.6a, b). In contrast, sizes of emerging beetles were similar within the tree, although beetles showed a trend for being larger in the upper parts of the stem (maxT = 2.03, p = 0.10, N = 12, Fig. 1.6c, d)

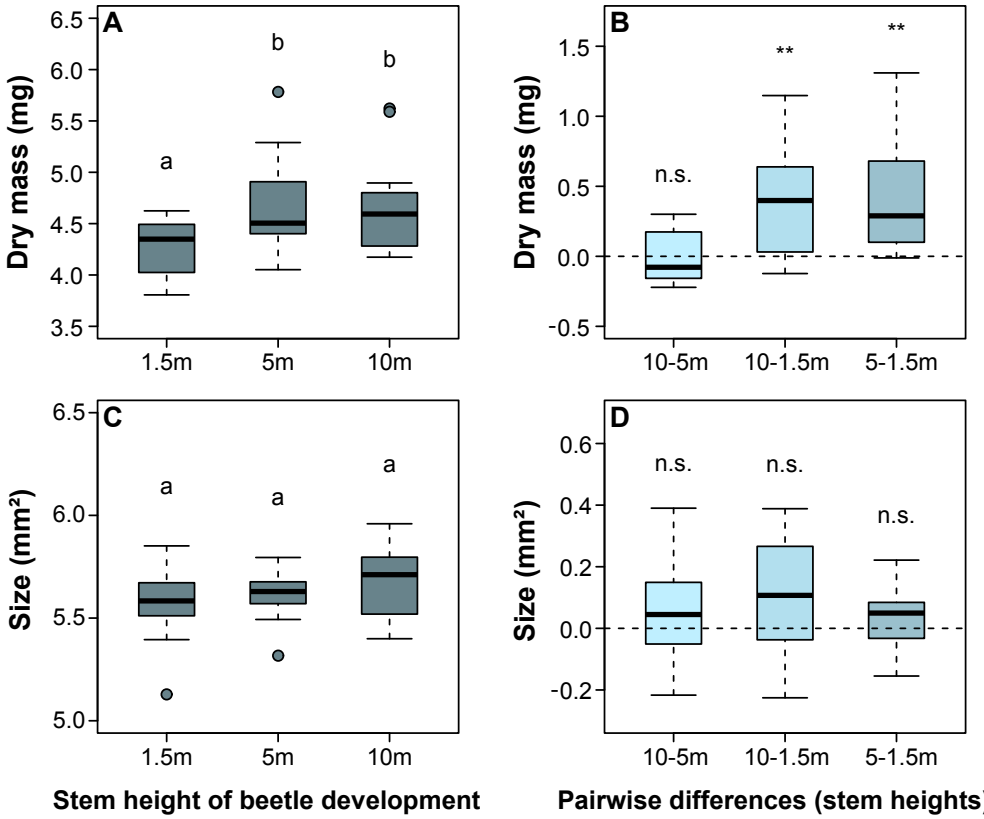


**Fig. 1.6.** Comparison of fitness parameters of beetles developing at different stem heights. **A.** Dry masses of beetles which developed at 1.5, 5 and 10 m stem height and **B.** their respective pairwise differences, **C.** sizes (calculated as product of elytra length and pronotum width) at the three heights and **D.** their respective pairwise differences. Differing letters indicate significant differences. Significance levels are given as ** $p < 0.01$ and n.s. = not significant (Approximative General Independence Test, WNDT post-hoc test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

### 1.3.4 Fitness parameters of beetles with differing nutritional state simulated by starvation

Fresh masses, dry masses and lipid reserves decreased over the starvation period. At least on the fourth day of starvation these parameters were significantly lower than directly after emergence from brood logs (Approximative K-Sample Permutation test, NDWD post-hoc test: all maxT > 2.85; all $p < 0.03$, all N = 100, Fig. 1.7a, b and c). In contrast, flight distances were not significantly different between durations of starvations (maxT = 2.22, $p = 0.17$, N = 50, Fig. 1.7d).

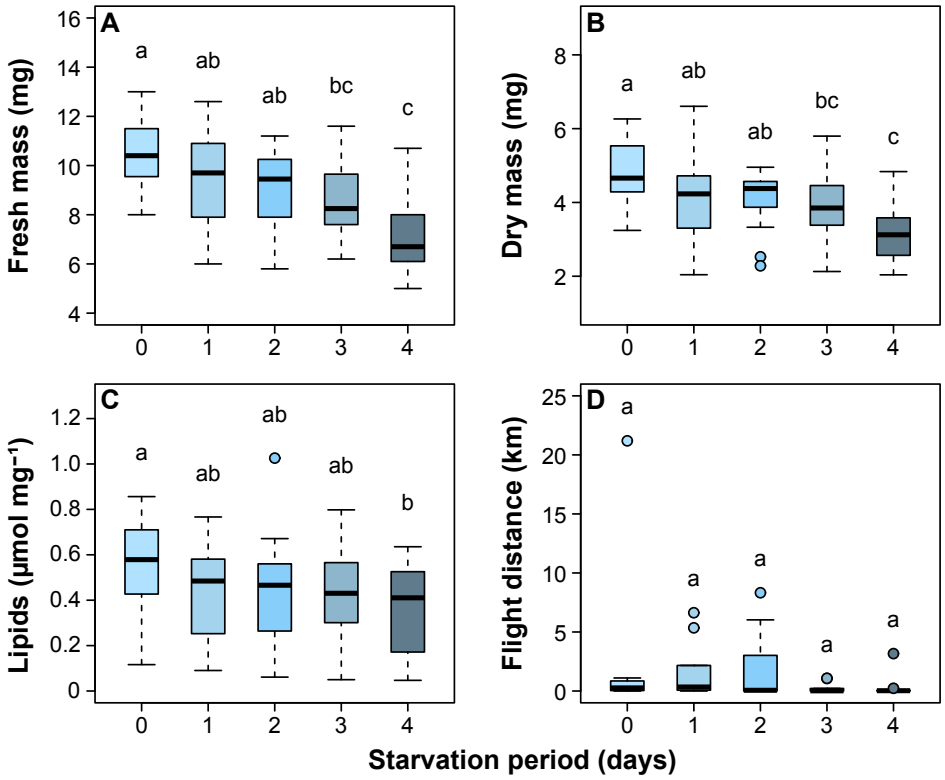


**Fig. 1.7.** Comparison of fitness parameters after different periods of starvation: **A.** fresh masses, **B.** dry masses, **C.** lipids as acylester equivalents per dry mass and **D.** flight capacity. Differing letters indicate significant differences (Approximative K-Sample Permutation test, NDWD post-hoc test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

## 1.4 Discussion

The results of this chapter suggest that an initial infestation at higher stem heights should be favoured by *I. typographus*. There, nutrients (i.e. starch, glucose and sucrose) were significantly higher concentrated than in the phloem of the lower stem part. Moreover, total phenol and monoterpene concentrations were significantly lower (Tab. 1.1 and Fig. 1.3). Although *I. typographus* requires host monoterpenes for pheromone production (Blomquist et al., 2010), they have to expend energy reserves for their detoxification (Gatehouse, 2002; Gries et al., 1990; Raffa et al., 2005; Reddemann and Schopf, 1996). Additionally, mortality caused by terpenoids can be significant. Raffa et al. (2005) estimated that constitutive terpenoid concentrations in the red pine can kill 60% of adults within two days and induced concentrations 90% within three days. Therefore, tree sectors with low concentrations of terpenoids should be favoured by pioneer beetles. Artificial girdling even amplified the effect of inhomogeneous metabolite distribution. Starch and sucrose concentrations were significantly lower below the phloem disruption (Tab. 1.2 and Fig. 1.5). In contrast, glucose

and fructose concentration were higher below the girdling. But their absolute concentrations were more than one magnitude lower than those of starch and sucrose. Thus, in comparison to the two latter they may play only a minor role as nutrients for *I. typographus*. Although phenolic and monoterpene concentrations were not significantly different, both showed a trend for being higher below the girdling. Additionally, in comparison to untreated trees, all studied nutrients, except for protein amino acids, were significantly less concentrated below the girdling, whereas studied toxic compounds showed no difference in this respect (Tab. 1.3). Since larval feeding activity results in a phloem girdling effect which considerably decreases nutrient transport (Franceschi et al., 2005; Postner, 1974), parental beetles should favour oviposition in higher stem regions. Because, the less brood systems are above their offspring, the higher their fitness advantage will be.

The observed metabolite distribution within spruces seemed to translate into beetle fitness. Beetles which emerged from the lower part of stems were significantly more lightweight (Fig. 1.6). Size of beetles did not differ within the stem, although it also showed a trend for being smaller at lower stem parts. The discrepancy of the effect on weight and size may be due to the fact that adult size is determined by pupal weight and thereby by conditions of larval development. Due to phloem senescence in the course of the infestation process, callow adults may experience conditions where the nutritional value of phloem is lower than during their larval stage. Thus, during maturation feeding, beetles may not be able to gain the optimal nutritional state which corresponds to their size due to depletion of resources. This hypothesis is supported by the findings of Riedinger et al (2009). In a choice experiment, they observed that beetles which were offered phloem of infested spruces with reduced sucrose content fed the same absolute amount as beetles offered phloem of non-infested spruces. Thus, energy uptake and as a result weight is reduced in beetles feeding on degenerated substrate.

The finding of decreasing offspring fitness with decreasing nutritional content was strengthened in the starvation experiment, in which weight and lipid reserves decreased with increasing time of starvation (Fig. 1.7). However, decreasing weights and lipid reserves did not affect flight capacity, as for example proposed by Gries (1985). This discrepancy between energy reserves and flight capacity as function of developmental conditions will be discussed in detail in the following chapters.

# Chapter 2: Influence of population density on beetle fitness

## 2.1 Introduction

The aggregation pheromone mediated mass attack of *I. typographus* can lead to high colonisation densities within trees. The resulting intraspecific competition among larvae might be a major factor in determining fitness of surviving offspring. When a tree is colonised beetles do not position their brood systems randomly (Byers, 1984) and larvae have little ability to cross consumed bark areas (de Jong and Grijpma, 1986). This avoidance strategy leads to exploitation competition in high population densities, because the expansion of larval galleries is limited if brood systems are packed densely. It has been shown numerously that weight, size and fat content of bark beetle progeny decreases with increasing colonisation density. Atkins (1975) reported that dry weight and lipid reserves of the Douglas-fir beetle *Dendroctonus pseudotsugae* correlates negatively with attack density. Botterweg (1983) found the same relationship, i.e. a negative influence of population density on size and lipid content, in *I. typographus*. Also Sallé et al. (2005) reported that beetles in epidemic population conditions were smaller than those in endemic conditions. Beside the limitation of space and availability of food, faster deterioration of bark through dense colonisation may additionally reduce fitness of the offspring. To this effect, Botterweg (1982) reported a declining lipid content of beetles with progressing emergence time. The reduced fitness traits in high population densities may contribute to decrease colonisation success and decelerate population growth. Low lipid reserves for instance may negatively influence host selection behaviour (e.g. Elkin and Reid, 2010; Reid and Purcell, 2011; Sallé and Raffa, 2007), pheromone production (e.g. Anderbrant et al., 1985; Birgersson et al., 1988), the ability to detoxicate secondary host tree metabolites (e.g. Gatehouse, 2002; Gries et al., 1990; Raffa et al., 2005) or fecundity (e.g. Amezaga and Garbisu, 2000; Elkin and Reid, 2005; Reid and Roitberg, 1995) of the progeny. Furthermore, several authors argued that the amount of lipid reserves in bark beetles is related to their dispersal capacity (e.g. Anderbrant and Schlyter, 1989; Anderbrant et al., 1985; Gries, 1985; Wallin and Raffa, 2004; Williams and Robertson, 2008). Some concluded that high densities would therefore negatively influence population dynamics and, as negative feedback, eventually contribute to the decline of outbreaks (e.g. Anderbrant and Schlyter, 1989). However, other authors did not find such correlations. For

example, Botterweg (1982) found no influence of sex, size or fat content on dispersal capacity and Forsse and Solbreck (1985) found no influence of elytra length on flight duration.
It has to be stressed that flight capacity cannot be directly translated into dispersal distances because in the absence of wind drift, tree cues and aggregation pheromones, *I. typographus* dispersal is assumed as non-linear process (e.g. Byers, 1996; 2000; Franklin and Gregoire, 1999). However, knowledge of flight capacity is fundamental for determining dispersal and, as a result, spatial distribution of subsequent infestations. Therefore, in this chapter fitness parameters (i.e. weight, size, lipid reserves and flight capacity) were compared between individuals that developed in high and low population densities.

## 2.2 Material and Methods

*Experimental design*

*I. typographus* stocks with two different population densities were established: (a) 50 brood systems per square meter (low density) and (b) 500 brood systems per square meter (high density). Light conditions were 16:8 (L:D) and temperature was between 20 and 25°C. Beetles originated from the seventh generation of the laboratory stock of the Institute for Animal Ecology (see chapter 1.2.4). 499 brood systems per square meter were found to be the average colonisation density across four outbreak years by Weslien and Regnander (1990) and thus considered to represent high population density. Optimal density was described as 500 maternal galleries per square meter bark (Schopf and Köhler, 1995), which would mean 250 brood systems when a male averagely mates with two females. The desired population densities were achieved by placing four beetles each in pieces of silicone tubes attached to spruce logs (Fig. 2.1). Therefore, number and positions of brood systems were controlled.

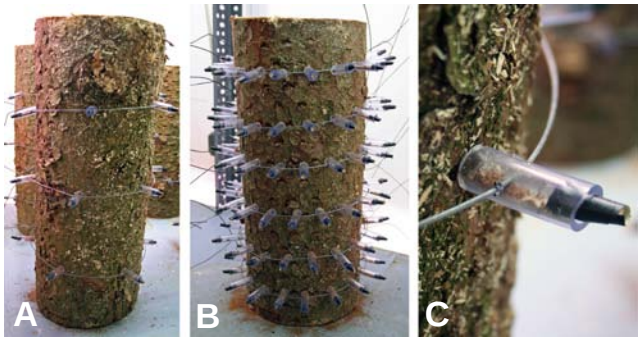


**Fig. 2.1.** Laboratory stocks on spruce logs with differing *I. typographus* population densities. **A.** 50 brood system per square meter bark (low density) and **B.** 500 brood systems per square meter (high density). **C.** Locations of brood systems were controlled by insertion of four individuals in each piece of silicone tube.

Fresh and dry masses, sizes (as product of length and pronotum width) and lipid reserves as acylester equivalents (see chapter 1.2.4 for detailed methods) of 96 beetles from each population density were compared directly after emergence from the logs. Additionally, flight capacity of 36 beetles from each population density was tested using automated flight mills (see chapter 1.2.4 for detailed methods). Furthermore, heterozygosity across twelve microsatellite loci of 35 beetles tested in flight mills from each brood density was analysed.

*Microsatellite analysis*

Microsatellite analysis was conducted by Bernhard C. Stoeckle (Molecular Zoology, Department of Animal Science, Technische Universität München).

Genomic DNA was isolated from bark beetles lysate according to Stoeckle et al. (2010). For PCR the twelve microsatellite loci GAA3F10, GAA5D8, GT1B6, GT343, GAA4C3 (Sallé et al., 2003), ITY84 (Stoeckle et al., 2010), ITY12, ITY19, ITY22, ITY25, ITY26 and ITY28 (Stoeckle and Kuehn, 2011) were used. The fluorescently labelled (6-FAM, HEX and TAMRA) PCR products were separated on 6% polyacrylamide gels on an ABI Prism 377 automated sequencer (Perkin Elmer, Waltham, Massachusetts, USA) and scored by reference to a ROX standard (79–540 bp) by GENESCAN® 3.1.2 and GENOTYPER® 2.5 software (Applied Biosystems, Foster City, CA, USA).

*Statistical analysis*

Fresh and dry masses, sizes, lipid reserves per dry mass and flight distances were compared between the two population densities using Mann-Whitney U-tests. Furthermore, beetle sizes and fresh masses were correlated with flight distances of the respective beetles with Spearman rank correlation tests. Standardised heterozygosity (Coltman et al., 1999) was computed for 35 individuals from each population density using the R extension ‘Rhh’ (Alho et al., 2010) and correlated to the respective flight distances with Spearman rank correlation tests. All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011).

## 2.3 Results

Fresh and dry masses, sizes and lipid reserves per dry mass after emergence from brood logs of beetles from low population density were significantly higher than those of beetles from high densities (Mann-Whitney U-tests: all $Z > 2.67$, all $p < 0.01$, all $N = 192$, Fig. 2.2a-d). Flight distances did not differ significantly between beetles from the two population densities ($Z = 0.82$, $p = 0.42$, $N = 72$, Fig. 2.2e). The maximal flight distance accomplished by a beetle was 26.9 km. The median flight distance of beetles from low densities was 5.7 km, and that of beetles from high densities was 3.6 km. In contrast to directly after emergence, lipids were not any more significantly different between beetles from the two population densities ($Z = 0.45$, $p = 0.67$, $N = 57$, Fig. 2.2f) and not normally distributed after flight, but in large parts depleted (compare Fig. 2.2d and f).

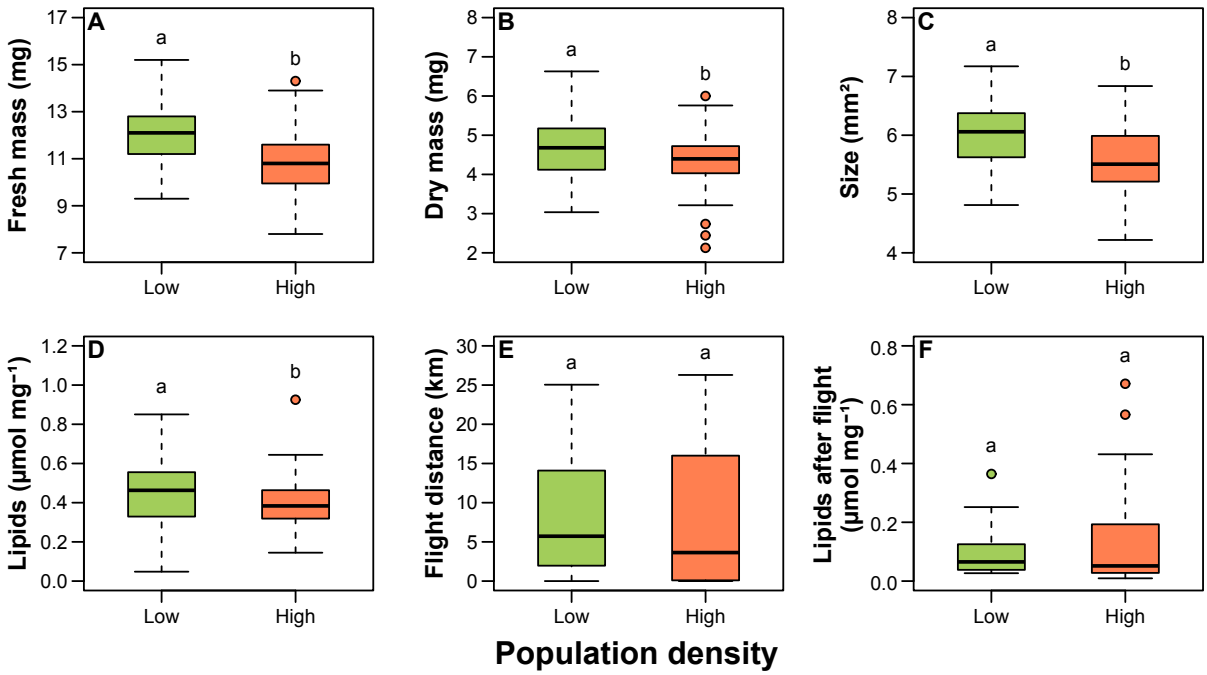


**Fig. 2.2.** Fitness parameters of beetles from low and high population densities. **A.** fresh mass, **B.** dry mass, **C.** size as product of elytra length and pronotum width, **D.** lipid reserves as acylester equivalents per dry mass, **E.** flight capacity and **F.** lipid reserves after flight as acylester equivalents per dry mass. Differing letters indicate significant differences (Mann-Whitney U-tests). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

For the following analyses beetles from both population densities were pooled, since flight distances did not differ significantly between them (see Fig. 2.2e). Neither beetle size nor fresh masses showed strong correlations with the achieved flight distances ($R^2_{size} = 0.04$, $R^2_{mass} = 0.09$), although the correlation between fresh mass and flight distance was statistically significant (Spearman rank correlations, size: $r_S = 0.20$, $p = 0.09$, $N = 72$, fresh mass: $r_S = 0.30$, $p = 0.01$, $N = 72$, Fig. 2.3a, b). Furthermore, standardised microsatellite heterozygosity did not correlate with flight capacity ($r_S = 0.20$, $p = 0.10$, $N = 70$, Fig. 2.3c).

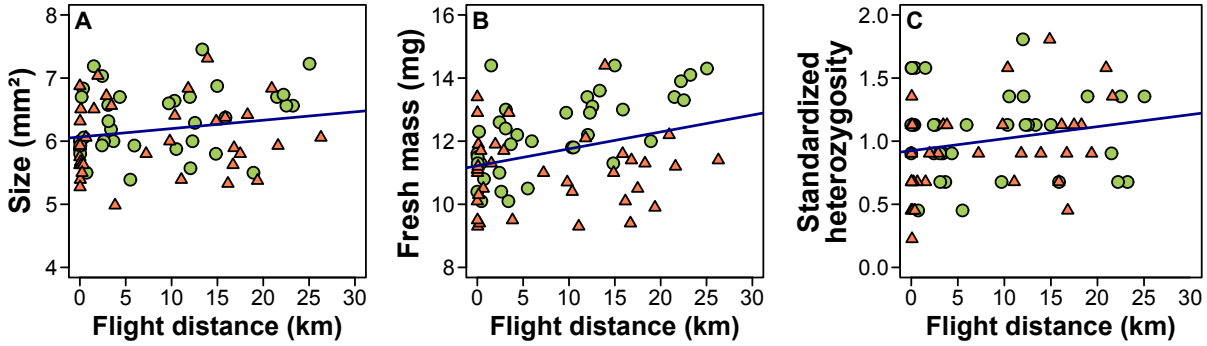


**Fig. 2.3.** Relationship between flight capacity and fitness parameters. **A.** Beetle size as product of elytra length and pronotum width, **B.** fresh mass and **C.** standardised heterozygosity. Circles = individuals from low density, triangles = individuals from high density (pooled for regression analysis).

## 2.4 Discussion

The results of this experiment confirm that increased population density has a negative influence on fitness parameters like fresh and dry mass, size and also lipid reserves (Fig. 2.2; Atkins, 1975; Botterweg, 1983; Williams and Robertson, 2008). However, flight capacity was not differing in individuals between low and high population densities in this study. Moreover, there was no correlation between beetle weight or size and flight capacity (Fig. 2.3). These results strengthen the findings of Botterweg (1982), Forsse and Solbreck (1985) or Jactel (1993) and contradict the common hypothesis that the amount of lipid reserves is directly linked to flight capacity in bark beetles (e.g. Anderbrant, 1985; Anderbrant and Schlyter, 1989; Gries, 1985).

Moreover, no correlation between flight capacity and microsatellite heterozygosity was found (Fig. 2.3). Generally, increased heterozygosity is assumed to reflect higher fitness (Chapman et al., 2009), because it is considered to support coping with adverse or stochastic environmental conditions ('episodic heterozygote advantage': Samollow and Soulé, 1983). On

an individual level, three hypotheses have been proposed to explain significant heterozygosity-fitness correlations, i.e. estimates of the relationship between genetic diversity and life-history, morphological and physiological traits (reviewed in Hansson and Westerberg, 2002). In case analysed markers are expressed and have an effect on fitness themselves (e.g. allozyme or major histocompatibility complex loci), they may be under direct selection and functional overdominance may occur ('direct effect hypothesis', reviewed in David, 1998). However, microsatellite markers are usually selectively neutral, yet their genetic diversity often is correlated with fitness (Goldstein and Schlötterer, 1999). In the 'local effect hypothesis', neutral marker heterozygosity-fitness correlation is explained by non-random association of neutral loci with fitness affecting loci, i.e. they are in 'linkage disequilibrium' (reviewed in Chapman et al., 2009; David, 1998). The 'general effect hypothesis' explains correlations of neutral markers with fitness traits by non-random association of diploid genotypes in zygotes. It reflects genome-wide fitness cost of homozygosity, i.e. neutral marker and fitness loci are in 'identity disequilibrium' (reviewed in Chapman et al., 2009; David, 1998). On the basis of a 'general effect', it would have been expected that long range fliers would be more heterozygous than short range fliers in the present study. However, the number of sampled individuals (70) and loci (12) most probably was too low in this study to find a robust heterozygosity-fitness correlation, if existent. A minimum of 600 individuals is suggested to be needed for robust conclusions (Coltman and Slate, 2003). Additionally, with 12 markers only, it is more likely that the found relationship represents marker-fitness correlation rather than genome-wide heterozygosity-fitness (DeWoody and DeWoody, 2005).

Nevertheless, the depletion of lipid reserves within 24 hours of tethered flight was considerably higher than in a comparable time of starvation (compare Fig. 2.2d, f and Fig. 1.7c). A depletion of lipid reserves after flight was also reported for *I. typographus* (e.g. Birgersson et al., 1988) and for *D. pseudotsugae* (e.g. Thompson and Bennett, 1971; Williams and Robertson, 2008) in earlier studies. Therefore it is reasonable that lipids play a major role as fuel for flight in bark beetles. Flight capacities of more than 25 km, as found in this study, are not unusual in single scolytid specimens. For example, *Ips sexdentatus* was found to be able to cover distances up to 50 km (Jactel and Gaillard, 1991), *I. confusus* up to 31 km (Kinn, 1971), *I. pini* more than 20 km (Robertson and Roitberg, 1998) and *D. pseudotsugae* more than 30 km (Atkins, 1961). High flight capacity enables individuals to locate resources that are spatially scattered, like weakened host trees. On the other hand, especially in species which require a certain number of attacking individuals, dispersing far from a source

decreases spatial density of individuals, as suggested by Anderbrant and Schlyter (1989, Fig. 5). Thus, colonisation success would be decreased, which might explain why short flight distances are favoured, in large parts independent of energy reserves.

Although lipid content decreased considerably during flight activity, individuals never consumed their complete reserves. The reduced fitness by intraspecific competition may rather be mediated by reduced energy reserves and manifest in reduced colonization success through reduced ability for pheromone synthesis (e.g. Byers, 1989; Gries et al., 1990), reduced ability for detoxification of host tree compounds (e.g. Gries et al., 1990; Wallin and Raffa, 2000) or reduced oviposition (e.g. Elkin and Reid, 2005). Especially in females, size and lipid content may have a significant influence on reproductive fitness because energy for egg production may be missing. Komonen et al. (2011) reported that in a three times higher colonisation density reproductive success in terms of offspring production was reduced by one fourth. Thus, population growth may rather be limited by competition for space and reduced energy reserves of offspring than by reduced dispersal capacity.

# Chapter 3: Influence of overwintering site and time of hibernation onset on population structure and beetle fitness

Parts of this chapter were published in:

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## 3.1 Introduction

Winter mortality can drastically modulate *I. typographus* population dynamics. Depending on infestation intensity, latitude and elevation, populations can be reduced up to 50% during winter (Biermann, 1977; Dworschak et al., 2009; Faccoli, 2002). In Scandinavia, mortality of even 100% of individuals overwintering in their host tree was reported (Annila, 1969). One important mortality factor during winter is temperature. In Scandinavia, winter temperatures can fall well below -30°C, which is lethal for *I. typographus* (Annila, 1969; Koštál et al., 2011). Generally, insects can increase cold hardiness by upregulating cryoprotectants like glycerol or several heat shock proteins (Hahn and Denlinger, 2011). *I. typographus* protects itself against chilling injuries by behavioural patterns such as gut evacuation, and additionally by accumulation of a complex mixture of cryoprotectants consisting of glycerol, several other polyols and sugars (Koštál et al., 2011; Koštál et al., 2007). Additionally, adult beetles overwintering in their host tree usually move to the rather dry outer parts of the bark and thus are protected against inoculative freezing through external ice crystals (Doležal and Sehnal, 2007a; Koštál et al., 2011; Schopf and Kritsch, 2010). In contrast, larvae and pupae are tied to the rather moist phloem which might drastically increase their mortality risk by inoculative freezing (Koštál et al., 2011). Duration of critical temperatures greatly influences the extent of lipid reserve utilization for increasing cold hardiness (Hahn and Denlinger, 2007), and thus minimizes reserves needed for dispersal, host tree colonisation or reproduction after overwintering. In the first part of this chapter, winter mortality and post-diapause fitness along an altitudinal, and thereby temperature, gradient was studied in an experimental setup in the Bavarian Forest National Park (Germany). Fitness was measured as body weight, lipid content and flight capacity.

Not only elevation or latitude (and thereby temperature), but furthermore hibernation site – be it within the tree or in the soil litter – might have a severe impact on winter mortality and also

post-diapause fitness. Earlier studies showed that absolute minimal temperatures and also temperature dynamics can be drastically different between the bark of standing trees and in the soil underneath the snow cover (Annila, 1969; Bakke, 1968; Leather et al., 2008). Whereas bark temperatures of infested (and usually dead) trees fluctuate with surrounding air temperatures, temperature beneath a constant snow cover is stable and does not usually fall below the freezing point (Annila, 1969; Bakke, 1968). Therefore it was postulated that *I. typographus* hibernates mainly in the soil litter. Across Scolytinae, there is no general pattern of overwintering site. For example, *Ips acuminatus* (Gehrken, 1984), *Dendroctonus frontalis* (Lombardero et al., 2000a) or *Trypodendron laeve* (Martikainen, 2000) stay in their host trees for overwintering, whereas *Ips pini* (Lombardero et al., 2000a), *Tomicus piniperda* (Salonen, 1973) or *Trypodendron lineatum* (Zumr, 1983) move to the soil litter.

Annila (1969) observed that a large fraction of the population in his study area in Norway left their host tree in late autumn. However, in this study it remained unclear whether these beetles moved to the soil litter or bored in elsewhere in a tree. Nevertheless, a number of other studies supported this hypothesis. Franz (1948a; b) observed up to 4,000 individual per square meter in the soil litter. But he sampled near a timber deposit in a sanitation felling area, so that it is questionable if he observed a natural or man-made phenomenon. Also Schneider-Orelli (1947), Kuhn (1949) and Botterweg (1982) observed that *I. typographus* is hibernating predominantly in the soil litter. Komonen et al. (2011) and Hrasovec et al. (2011) recently found a ratio of soil and tree overwintering beetles of approximately 50:50 in Sweden and in Croatia, respectively. In contrast to these findings, Biermann (1977) observed that in Lower Saxony (Germany) less than 10% of beetles were hibernating in the soil. This was confirmed by Zumr (1982; between 0.6 and 4.7% of beetles hibernating in the soil) in the Czech Republic, Onysko and Starzyk (2011; less than 30% in the soil) in Poland and Harding and Ravn (1985; less than 30% in the soil) in Denmark. Across these studies a rough pattern emerged, whereupon beetles of northern latitude or higher elevation tend to overwinter in the soil litter (but compare the more recent Scandinavian studies of Harding and Ravn, 1985 and Komonen et al., 2011). Beetles of more southern latitude and lower elevation tend to overwinter in their host trees. A similar pattern has been observed in *Ips grandicollis* in North America, where individuals of more southern populations remained under the bark and those of northern populations overwintered in the soil (Lombardero et al., 2000a). The discrepancy of overwintering location between Scandinavian and Central European populations might be due to differences in winter temperatures, which usually do not fall below the super-cooling point of -20 to -30°C of *I. typographus* (Annila, 1969; Koštál et al., 2011) in Central Europe.

Therefore beetles of southern latitudes might not be forced to leave their host trees to avoid chilling injuries caused by long time spans of extreme cold temperatures. But also in Central Europe results were mixed, and already Bender (1948) and Wild (1953) reported that beetles had left their host trees between September and December.

Based on these mixed results and the personal observation that bark beetle infested spruces lose a variable fraction of their bark during winter (also already noted by Biermann, 1977), depending on infestation density, exposition or weather conditions, the second part of this chapter aims to explain the underlying mechanism of soil overwintering. Using soil emergence traps in an infestation area in the Bavarian Forest National Park, following hypotheses were tested: (1) The primary overwintering location of *I. typographus* is the host tree. (2) Soil hibernation of *I. typographus* is mediated passively through falling bark. (3) Soil hibernating beetles remain mainly in the fallen bark and do not move deeper into the soil. (4) Soil hibernation implicates no post-diapause fitness advantage compared to hibernation in the host tree (fitness measured as body weight, lipid content and flight capacity).

Finally, in the third part of this chapter the time of hibernation onset was studied. It is commonly accepted that voltinism of *I. typographus* is mediated through temperature and especially photoperiod (Doležal and Sehnal, 2007a; Schopf, 1985; 1989). These studies showed that individuals remain in their brood systems when the day length decreases below 16 hours of light (between mid and end of August in Central Europe), and/or temperatures fall below the lower developmental threshold. As a result, populations in Scandinavia and at higher elevations are univoltine with only rarely occurring partial second generations (Annila, 1969; Austara et al., 1977; Botterweg, 1982; Faccoli and Bernardinelli, 2011; Forsse, 1991), whereas Central European lowland populations have two (Faccoli and Bernardinelli, 2011; Faccoli and Stergulc, 2006; Wermelinger et al., 2012) and in extremely warm years might even have three generations (e.g. Wild, 1953). If it holds true that pre-imaginal life stages of *I. typographus* are at higher risk of winter mortality than adult beetles, it is crucial for individuals to complete development before winter onset. Consequently, in an environment where the end of the season is unpredictable, they have to decide between initiating hibernation and an additional reproductive cycle when environmental factors are possibly still in favour of the latter. Therefore, emergence behaviour was followed using tree emergence traps in the Bavarian Forest National Park and additionally using caged logs of naturally infested spruces.

## 3.2 Material and methods

### 3.2.1 Population structure and fitness after overwintering along a temperature cline

*Experimental design*

On July 19th 2007, Norway spruce logs were infested with *I. typographus* in the laboratory. Logs were approximately 0.6 m in length and 0.25 m in diameter. Beetles originated from the 8th generation of the laboratory stock of the Institute for Animal Ecology (see chapter 1.2.4 for details). After one week at room temperature, the logs were caged in aluminium gauze. Thus, escape and supervening of beetles was prevented and circulation of air and humidity was ensured. Logs were attached to the north side of standing spruce trees within the forest stand 4 m above the ground in the Bavarian Forest National Park (Fig. 3.1a). Three logs were exposed at each of three altitudes ('Schwellhäusl': 49°5'54.21" N 13°13'4.36" E, 700 m a.s.l., 'Höllhänge': 49°4'32.52" N 13°18'32.82" E, 900 m a.s.l. and 'Ruckowitzschachten': 49°6'8.41" N 13°16'40.03" E, 1100 m a.s.l., Fig. 3.1b). Additionally, three logs were exposed outside the Institute for Animal Ecology in Freising, Germany (48°24'0.05" N 11°43'0.28" E, 500 m a.s.l.). The experiment was repeated one month later as described above (initiation of brood systems on August 9th 2007 and exposure on August 18th 2007).

Air temperature was measured in hourly intervals in one of the aluminium gauze cages at each altitudinal level using Thermocron iButtons® (Maxim DS1922L, San José, CA, USA). Air temperature was considered to correspond to bark temperature since in cut logs a potential cooling effect of xylem water flow is eliminated and insolation was minimised by exposition within the forest stand. Thermal sums (in day degrees, dd) above the lower developmental threshold (LDT) were accumulated according to Wermelinger and Seifert (1998) to determine the developmental stages of beetles at each point in time throughout the experiment.

The logs were brought back to the laboratory in spring 2008, after snowmelt and before spring swarming at the respective altitudinal level (500 m: 29.04.2008, 700 m: 09.04.2008, 900 m: 07.05.2008, 1100 m: 20.05.2008). Emerging beetles before (beetles in aluminium gauze cages outside the logs) and after overwintering (beetles which emerged from the logs in the laboratory), dead beetles which remained in the brood systems and brood systems were counted. Flight capacity of 20 emerging beetles per exposure date and altitudinal level was tested using automated flight mills (see chapter 1.2.4 for detailed methods). Additionally, fresh and dry mass, size as product of elytra length and pronotum width and lipid content as acylester equivalents per dry mass were determined for 20 beetles per exposure and elevation immediately after their emergence from logs (except for the highest altitude of the second

exposure date where the number of emerging beetles was too low; see chapter 1.2.4 for detailed methods). Furthermore, genetic structure of 45 surviving beetles from the lowest altitude was compared to 49 beetles from the highest altitude using twelve microsatellite loci. Microsatellite analysis was conducted by Bernhard C. Stoeckle (Molecular Zoology, Department of Animal Science, Technische Universität München, see chapter 2.2 for detailed methods).

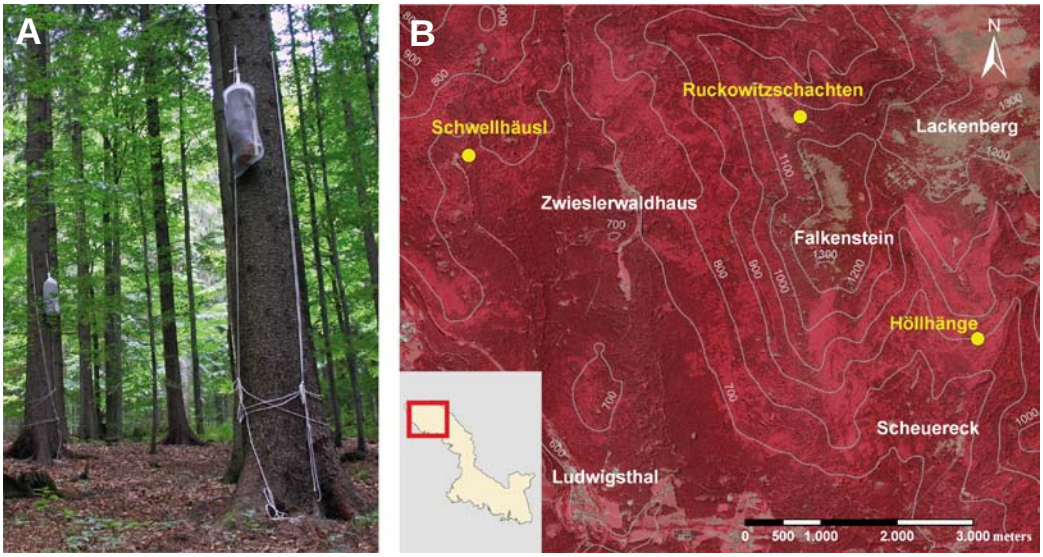


**Fig. 3.1.** Setup and sites of *I. typographus* infested spruce logs for overwintering along the altitudinal cline. **A.** Caged logs attached to standing trees within the forest stand after infestation with *I. typographus* in the laboratory. **B.** Aerial colour infrared image of exposition sites (yellow points) during winter in the Bavarian Forest National Park, Germany. Location within the park is shown on the generalised map in the bottom left corner. Aerial image with kind permission by Bavarian Forest

*Statistical analysis*

Accumulated effective thermal sums in day degrees (dd) for each altitude were calculated as '$\sum (\sum (T_{1\text{-}24} - LDT) / 24)$',

with '$T_{1\text{-}24}$' as hourly measured temperatures of one day and 'LDT' as the lower developmental threshold temperature of the respective developmental stage. LDTs of 10.6°C for eggs, 8.2°C for larvae, 9.9°C for pupae and 3.2°C for maturation feeding and effective thermal sums of 52 dd for the development of eggs, 204 dd for that of larvae and 58 dd for that of pupae, and 239 dd for maturation feeding were used (Wermelinger and Seifert, 1998).

Percentages of beetles which had emerged before and after winter and of dead beetles were calculated per exposure and altitude as the number of respective individuals divided by the sum of all individuals. For the first exposure, beetles had been expected to complete their

development before winter. To test the effect of decreasing temperature with increasing elevation on overwintering success, emergence per brood system after winter was calculated for the different altitudes. Numbers of emerged and dead imagines after winter were compared between individuals which completed maturation feeding before winter and all other developmental stages using a chi-square test of homogeneity. Fresh and dry mass, size, lipid content per dry mass and flight capacity of beetles was compared between the four altitudes using an Approximative K-Sample Permutation test with 10,000 Monte-Carlo replications, followed by a Nemenyi-Damico-Wolfe-Dunn (NDWD) post-hoc test (for details see Hollander and Wolfe, 1999). Therefore, the 'oneway_test' function within the package 'coin' (Hothorn et al., 2009) of the R2.14.1-system (R Development Core Team, 2011) was used. Across both exposures, it had been expected that at least beetles of the second exposure at the higher altitudes cannot finish their development before winter. Therefore, overwintering success was tested in relation to the developmental stage during winter. Emergence per brood system was compared between logs where beetles had completed their maturation feeding before winter and all other logs using a Mann-Whitney U-Test. Fresh and dry mass, size, lipid content per dry mass and flight capacity were compared between beetles which had completed their maturation feeding before winter and all other beetles using Mann-Whitney U-Tests. All statistical analyses were conducted using R2.14.1 (R Development Core Team, 2011).
Pairwise $F_{ST}$ value (Weir and Cockerham, 1984) between surviving beetles from the lowest and the highest altitude was calculated with GENEPOP 4.1.4 (Rousset, 2008). Exact G test for significant population differentiation ($F_{ST}$) was also performed with GENEPOP 4.1.4 using 1,000 de-memorisation steps and 100 batches with 100,000 iterations per batch (Raymond and Rousset, 1995). Observed heterozygosity ($H_o$) and mean allele number per locus (MNA) were calculated for beetles of the lowest and the highest altitude with the extension 'Microsatellite Toolkit 3.1.1' (Park, 2001) for Excel (Microsoft Corporation 2007, Redmond, WA, USA).

### 3.2.2 Tree vs. soil overwintering: mechanism and influence on fitness

*Pilot study*

A pilot study was conducted at the plot 'Grandl' (GR) in the National Park Bavarian Forest, Germany (49°5'6.37"N 13°12'46.73"E, 750 m a.s.l.). In April 2008, 22 spruce trees, which had been infested by *I. typographus* in August 2007 and had lost only little of their bark during winter, were cut. From these trees 20 bark samples with a diameter of 6 cm and a distance of 50 cm each were taken. Additionally, 24 trees which had been infested in August

2007 and had lost most of their bark during winter were selected. 20 samples with a diameter of 6 cm were taken from the bark on the ground around the stem of each of these trees. Bark samples of one tree were put together in one eclector each and transferred to the laboratory. Emerging beetles were collected in a 50% v/v ethylene-glycol/water solution (Overlack AG, Mönchengladbach, Germany) at room temperature (20-22°C).

The number of emerging beetles per square meter bark did not differ significantly between bark collected from the ground and bark taken from the stem (t-test: $t = 1.46$, $p = 0.15$, $N = 46$, Fig. 3.2). This result indicated that *I. typographus* does not leave its host tree for soil hibernation and that its primary overwintering location may be in the bark of standing trees. Thus, beetles would reach the soil only via falling bark during winter. To validate this hypothesis, the experiment described in the following section was conducted.

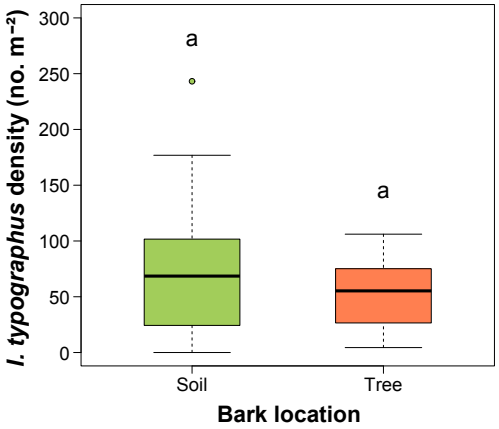


**Fig. 3.2.** Number of emerging *I. typographus* per square meter bark collected from soil and stems after winter. Identical letters indicate no significant differences (t-test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

*Experimental design*

The main study was conducted in April 2009 in the Bavarian Forest National Park (GR, 49°5'6.37" N 13°12'46.73" E). Spruces in a bark beetle outbreak area were selected and classified in following categories: (a) seven trees which were infested by *I. typographus* in August 2008 and had lost most of their bark during winter (> 50%, Fig. 3.3a), (b) five trees which were infested in August 2008 and had lost little bark (< 50%, Fig. 3.3b) and (c) three non-infested trees as control (Fig. 3.3c). Additionally, (d) two trees which were infested in May 2008 and had lost more than 50% of their bark and (e) two trees which were infested in May 2008 and had lost less than 50% of their bark were selected (Fig. 3.4).

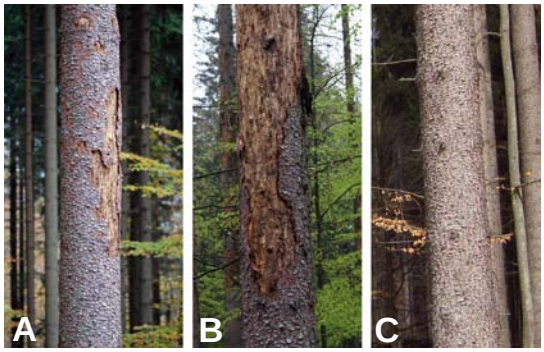


**Fig. 3.3.** Selected tree categories at which soil emergence traps were set up. **A.** infested trees with less and **B.** with more than 50% fallen bark after winter. **C.** Non-infested trees.

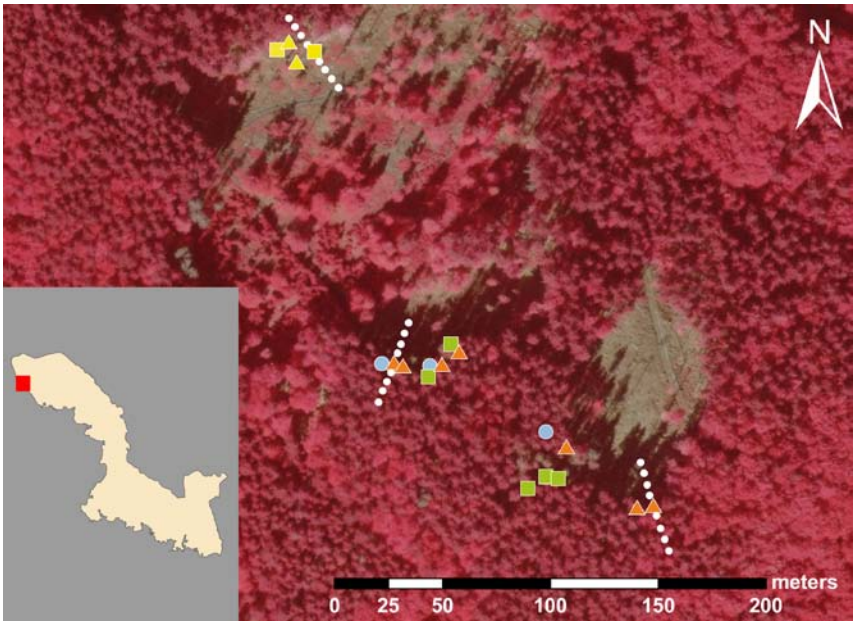


**Fig. 3.4.** Sites of soil emergence traps for trapping overwintered *I. typographus*. Triangles = four traps at trees with more than 50% fallen bark, squares = two traps at trees with less than 50% fallen bark, blue circles = two traps at non-infested trees, white circles = gradient traps with distances of 5, 10, 15 and 20 m from infested trees, yellow = trees infested in previous spring, orange and green = infested in previous summer. Location within the Bavarian Forest National Park is shown on the generalised map in the bottom left corner. Aerial image with kind permission by Bavarian Forest National Park Administration, Grafenau.

At the base of these trees soil emergence traps were set up on April 15$^{th}$, directly after the snow cover had melted and before spring swarming (Fig. 3.5). The traps had a base area of 0.5 m$^2$. They consisted of a wire skeleton which strained a reversed cloth funnel. The cloth ensured circulation of air and humidity. The funnel mounted in a transparent container on top. The container contained a 50% v/v copper sulphate/water solution (Carl Roth GmbH, Karlsruhe, Germany) to which a few drops of Sakrotan® (Reckitt Benckiser, Parsippany, NJ, USA) were added to prevent fungal growth. At the base of non-infested trees and trees with less than 50% fallen bark (categories b, d and e) two traps were set up. One of the traps was

orientated towards the forest stand, and one towards the forest gap (previous infestation). At the base of trees with more than 50% fallen bark (categories a and c) four traps were set up. Again, one trap pair was orientated towards the forest stand and one towards the gap. Fallen bark was left below one trap and removed below the other per trap pair (Fig. 3.6).

The removed bark from each trap was transferred separately to eclectors in the laboratory, where emerging beetles were caught and counted. In total, 50 emerged individuals were used for fresh mass, dry mass and lipid content analysis and 40 to test flight capacity per overwintering site (i.e. soil and tree, see chapter 1.2.4 for detailed methods). Furthermore, bark samples from eight tree trunks were taken at 5 m height and were transferred separately to eclectors in the laboratory. Fresh mass, dry mass and lipid content of 50 and flight capacity of 38 emerging beetles were analysed. Additionally, fresh mass, dry mass and lipid content of 50 beetles which were collected from five trees before winter (November 2008) were analysed.

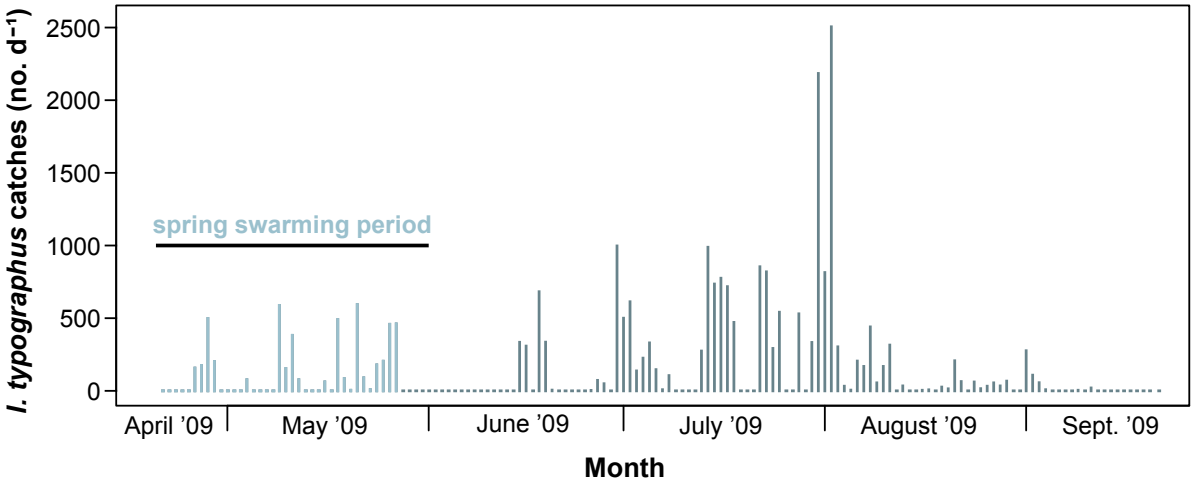


**Fig. 3.5.** Progression of *I. typographus* flight activity in 2009 on the plot 'Grandl', obtained by pheromone trap catches. Spring swarming period of parental beetles was between April, 25th and May, 26th. (data with kind permission by Gabriela Lobinger, Bavarian State Institute of Forestry, Freising).


**Fig. 3.6.** Setup of soil emergence traps. **A.** Trap consisting of a wire skeleton, a reversed cloth funnel and a container on top. **B.** Close-up view of the top container containing preservation fluid (ethylene glycol/water, 50% v/v) and soil emerged beetles. **C.** Trap pairs at the base of trees with more than 50% fallen bark. Below one trap of each pair the fallen bark was **D.** left and **E.** removed.

In addition to the traps at tree bases, gradients of traps with a distance of 5, 10, 15, and 20 m from three of the trees were set up in direction of the forest stand and forest gaps. Two of these central trees were infested in August 2008 and had lost more than 50% of their bark during winter and one was infested in May 2008 and had lost less than 50% of its bark. The traps were made of roofing cardboard and had a base area of 1 $m^2$.

Beetles in the traps were counted on June 4$^{th}$ 2009 after the main spring dispersal was finished (Fig. 3.5). The number of *I. typographus* was counted as individuals per square meter soil. The amount of bark under each trap was quantified as bark area in square meter per square meter soil.

*Statistical analysis*

The number of soil emerged beetles per square meter was compared between categories (a) - (c) using an Approximative K-Sample Permutation test with 10,000 Monte-Carlo replications, followed by a Nemenyi-Damico-Wolfe-Dunn (NDWD) post-hoc test (for details see Hollander and Wolfe, 1999). Therefore, the 'oneway_test' function within the package 'coin' (Hothorn et al., 2009) of the R2.14.1-system (R Development Core Team, 2011) was used. Additionally, the number of emerged beetles per square meter soil was correlated with the amount of bark per square meter soil using a Spearman rank correlation test. The number of emerged beetles was compared between trap pairs with left and removed bark with a paired-sample Wilcoxon signed rank test. Fresh masses, dry masses and lipid content per dry mass between beetles sampled from trees before winter, from trees after winter and from the soil after winter were compared using an Approximative K-Sample Permutation test, followed by an NDWD post-hoc test. Flight capacity of beetles which emerged from soil samples was compared to that of beetles which emerged from tree samples with a Mann-Whitney U-test. All statistical analyses were conducted using R version 2.14.1 (R Development Core Team, 2011).

### 3.2.3 Voltinism and time of hibernation onset

*Pilot study*

A pilot study was conducted at the plot 'Geissberg' in the National Park Bavarian Forest, Germany (48°55'47.08" N 13°20'39.01" E, 800 m a.s.l.). In April 2008, ten spruces which had been infested by *I. typographus* in May 2007 and ten trees which had been infested in August 2007 were selected. All twenty trees had lost only little of their bark during winter. From these trees twenty bark samples with a diameter of 6 cm and a distance of 50 cm each were

taken. All twenty bark samples of a tree were put together in one eclector each. Emerging beetles were collected in a 50% v/v ethylene-glycol/water solution (Overlack AG, Mönchengladbach, Germany) at room temperature (20-22°C).

Although the number of emerging beetles per square meter bark was considerably lower in trees which were infested in spring, it did not differ significantly from trees infested in summer (Mann-Whitney U-test: $Z = 1.79$, $p = 0.07$, $N = 20$, Fig. 3.7). This result indicates that not all *I. typographus* individuals of the first generation disperse in summer to start a second generation, as it would have been expected. To validate this hypothesis, the experiment described in the following section was conducted.

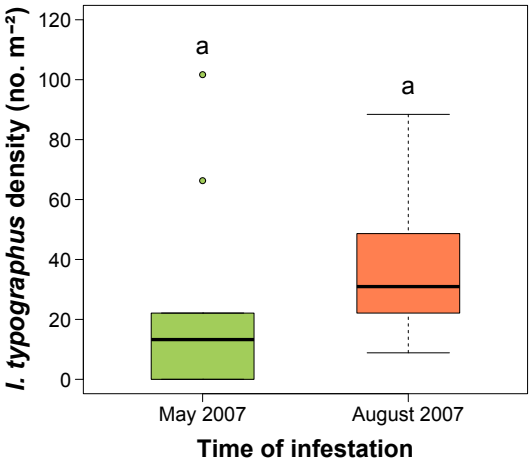


**Fig. 3.7.** Number of emerging *I. typographus* after winter from bark collected from tree trunks which had been infested in previous spring and summer. Identical letters indicate no significant differences (Mann-Whitney U-test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and

*Experimental design*

In June 2007, before emergence of filial beetles, ten spruces which had been infested by *I. typographus* in spring of the same year were felled on the plot 'Geissberg'. Three pieces of each trunk with a bark surface of approximately 0.6 m length and 0.3 m width were cut at circa 1.5, 5 and 10 m tree height. Those logs were put in one eclector each. Eclectors consisted of a plastic tube around the log with a plastic funnel at its bottom end. The funnel led into a container filled with water for trapping beetles. To reduce surface tension, few drops of detergent were added to the water. On top eclectors were sealed with a cloth gauze to ensure air and humidity ventilation (Fig. 3.8). The cut surface of logs was sealed with plastic film to minimise desiccation. Additionally logs were watered once a week. The self-contained configuration of eclectors prevented that beetles could escape and more importantly, that

additional beetles could enter the logs at a later point in time. Eclectors were kept outdoors under a roof on the institute area in Freising. The number of emerging beetles was counted weekly between June 2007 and June 2008. Dry weights and sizes as product of elytra length and pronotum width of a total of 3634 beetles were measured.

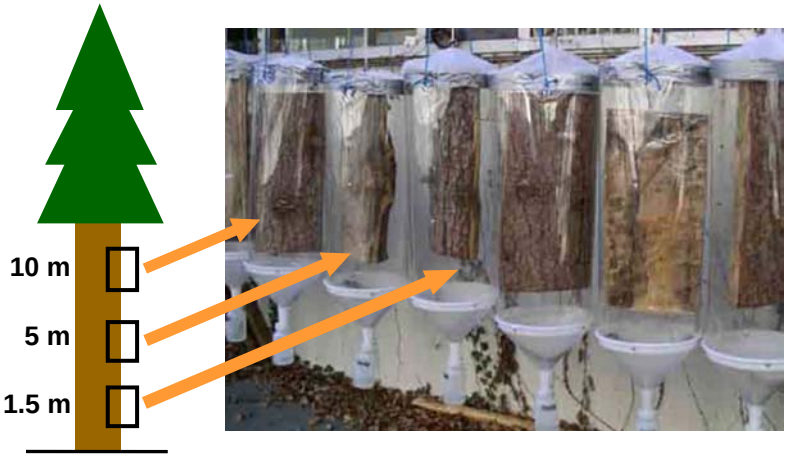


**Fig. 3.8.** Experimental setup used for following *I. typographus* emergence pattern: logs of three stem heights from infested spruces with eclector setup, consisting of a plastic tube, a plastic funnel at the bottom end with a trapping container for beetles, and cloth gauze on top.

The experiment described above was repeated in 2009, except that only logs from two tree heights were transferred to eclectors (1.5 and 10 m). The number of emerging beetles was counted weekly from June 2009 until June 2010. Twelve microsatellite loci of 200 emerging beetles were analysed by Bernhard C. Stoeckle (Molecular Zoology, Department of Animal Science, Technische Universität München, see chapter 2.2 for detailed methods). Beetles originated from five of ten trees, corresponding to 20 beetles per tree and year (2009 and 2010). Population structure, observed heterozygosity and mean allele number per locus were analysed and compared per tree between individuals emerged in 2009 and 2010. Air temperatures throughout both experiments was measured in hourly intervals using Thermocron iButtons® (Maxim DS1922L, San José, CA, USA).

In 2008, emergence pattern of first generation beetles was followed completely in the field. Therefore, six infested trees on each of the plots ‘Geissberg’ and ‘Grandl’ were equipped with passive traps in June 2008. At each tree three traps were set up at approximately 1.5 m, 5 m and 10 m stem height. They were constructed analogously to the eclectors described above, with the difference that only one half of the plastic tube was used. The opening of the half-tube was directed towards the stem, so that emerging beetles bumped against the plastic and were led into the container with preservation fluid through the funnel (see Fig. 1.1a). Bark

temperature throughout the experiment was measured in hourly intervals using Thermocron iButtons® (Maxim DS1922L, San José, CA, USA). Numbers of emerging beetles were counted weekly from June 2008 until October 2008 and from April 2009 until June 2009. Sizes of a total of 1415 beetles from the plot 'Geissberg' and 2026 beetles from the plot 'Grandl' were measured as product of pronotum width and elytra length.

*Statistical analysis*

The number of emerging beetles was calculated per square meter bark and week. Accumulated thermal sums in day degrees (dd) were calculated as described in chapter 3.2.1. Thermal sums were used to determine the point in time when beetles had finished maturation feeding, and thus were expected to emerge (calculation after Wermelinger and Seifert, 1998). Mean dry weights and sizes per trap were compared between the respective years of emergence with a paired-sample Wilcoxon signed rank test using the package 'coin' (Hothorn et al., 2008) of the R2.14.1 system (R Development Core Team, 2011). If more than 50% bark was fallen during winter at a trap, it was excluded from both years of this analysis.
Pairwise $F_{ST}$ values, observed heterozygosity ($H_o$) and mean allele number per locus (MNA) between beetles emerged in 2009 and 2010 per tree were calculated as described in chapter 3.2.1. Exact G test for significant population differentiation ($F_{ST}$) was performed with GENEPOP 4.1.4 using 1,000 de-memorisation steps and 100 batches with 100,000 iterations per batch (Raymond and Rousset, 1995). $H_o$ and MNA within trees were compared between beetles emerging in the two years with a paired-sample Wilcoxon signed rank test using the package 'coin' (Hothorn et al., 2008) of the R2.14.1 system (R Development Core Team, 2011).

## 3.3 Results

### 3.3.1 Population structure and fitness after overwintering along a temperature cline

At the lower two altitudinal levels, the first day with freezing temperatures occurred almost a month later than at the higher two. Accordingly, the last day below 0°C at the lowest elevation was over a month earlier than at the highest. Mean and minimal temperatures during this time period were comparable (Tab. 3.1, see also Fig. 3.9), both were considerably above the supercooling point of *I. typographus*, which is between -20 and -30°C (Annila, 1969; Koštál et al., 2011).

**Tab. 3.1.** Temperature parameters during the temperature cline experiment: first and last day below 0°C, number of days below 0°C, mean temperature and minimal mean daily temperature between first and last day below 0°C, and absolute minimal temperature.

| | 500 m a.s.l | 700 m a.s.l | 900 m a.s.l | 1100 m a.s.l |
|---|---|---|---|---|
| First day below 0°C | 2007/11/15 | 2007/11/10 | 2007/10/20 | 2007/10/19 |
| Last day below 0°C | 2008/03/06 | 2008/03/26 | 2008/04/07 | 2008/04/16 |
| No. of days below 0°C | 33 | 78 | 76 | 100 |
| Mean temperature (°C) | 2.35 | -0.57 | 0.31 | -0.44 |
| Minimal mean daily temperature (°C) | -5.05 | -7.78 | -7.53 | -9.96 |
| Absolute minimal temperature (°C) | -9 | -12 | -10.5 | -12 |

According to calculated accumulated effective thermal sums, beetles of both exposure dates at 500 m a.s.l. and beetles of the first exposure date at 700 and 900 m a.s.l. finished maturation feeding before temperatures fell below the lower developmental threshold. Beetles of both exposures at 1100 m a.s.l. and those of the second exposure at 700 and 900 m a.s.l. entered winter either as larvae or pupae. Nevertheless, the main emergence occurred after winter at temperatures above 20°C (Fig. 3.9). Across all elevations, only very few or no beetles at all had emerged before hibernation (between 0 and 3%, Fig. 3.10), even when maturation feeding was completed and temperatures above 16.5°C occurred (lower temperature threshold for *I. typographus* flight activity (Lobinger, 1994; compare Fig. 3.9a and Fig. 3.10). It remained unclear if the few beetles leaving the logs were filial beetles or parental beetles, which might have emerged in an early stage of the experiment for initiation of a second brood.

All dead imagines found in the bark were yellow, callow stages. Across all elevations and both exposure dates, mortality of imagines was significantly lower when individuals had completed maturation feeding before winter (chi-square test of homogeneity: $\chi^2 = 1943.36$, df=1, $p < 0.0001$, N = 5527 beetles with 4187 emerged and 66 dead when maturation feeding was completed and 667 emerged and 607 dead in all other developmental stages). Hibernation success in terms of percentage of emerged beetles was 97.82 ± 0.01% (mean ± s.e.) when beetles had completed maturation feeding before winter, and 61.38 ± 0.08% when larvae, pupae or young imagines were the predominant overwintering stages (beetles emerged before winter excluded, see Fig. 3.10).

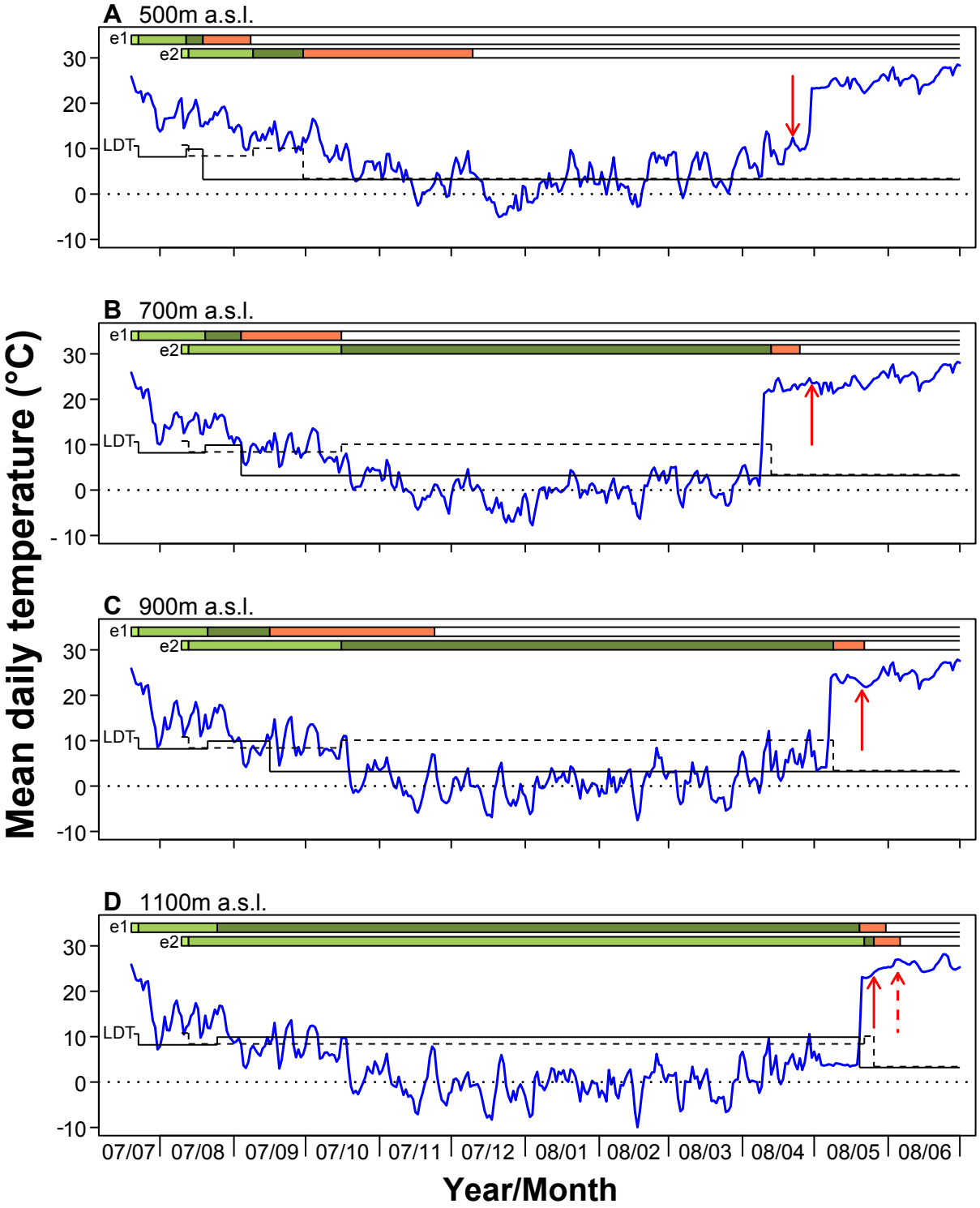


**Fig. 3.9.** Daily average temperatures and *I. typographus* development at the different elevations. Horizontal bars show developmental stages according to thermal sum calculation (after Wermelinger and Seifert, 1998): e1 = early exposure, e2 = late exposure; colouring from light to dark green = egg, larval, pupal stage; orange = maturation feeding (end of the orange bar corresponds to the time of calculated emergence). Arrows show real emergences from the logs, which coincide with calculated emergence for the three lower altitudinal levels. The solid line shows the lower developmental threshold (LDT) for the respective developmental stage of the early exposure, the dashed line for the late exposure (after Wermelinger and Seifert, 1998).

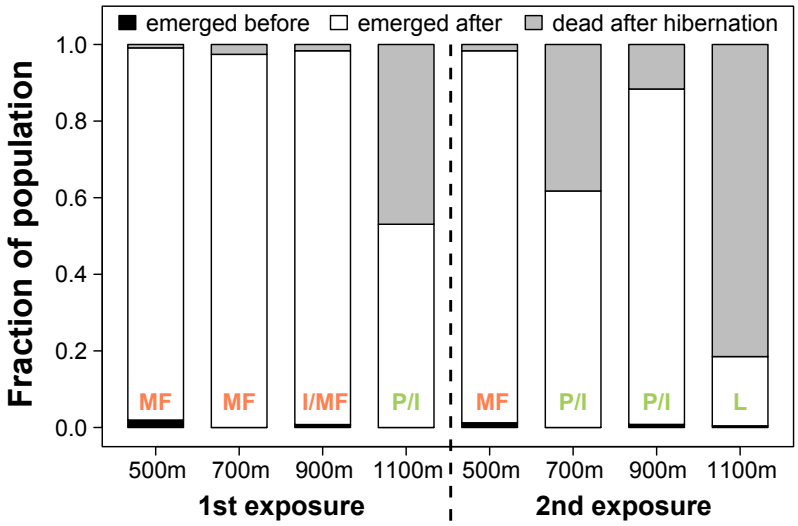


**Fig. 3.10.** Proportion of emerged *I. typographus* before (black) and after overwintering (white) and dead imagines (grey) for both exposure dates and all elevations. Fraction was calculated as average of the three exposed logs per exposure date and elevation. Letters indicate the calculated predominant developmental stage during winter (compare Fig. 3.9): L = larvae, P = pupae, I = callow imagines, MF = imagines with completed maturation feeding.

Across all exposed logs, 13.6 ± 1.3 (mean ± s.e.) brood systems per log were found. Emergence per brood system was not significantly different when considering the three lower elevations only, where maturation feeding was completed (Approximative K-Sample Permutation test: maxT = 0.65, p = 0.87, N = 3 logs per elevation). Across all elevations, emergence per brood system was significantly higher in those cases where maturation feeding was completed than in those where larvae or pupae overwintered (Mann-Whitney U-test: Z = 4.16, p < 0.0001, N = 24, Tab. 3.2 and Fig. 3.11).

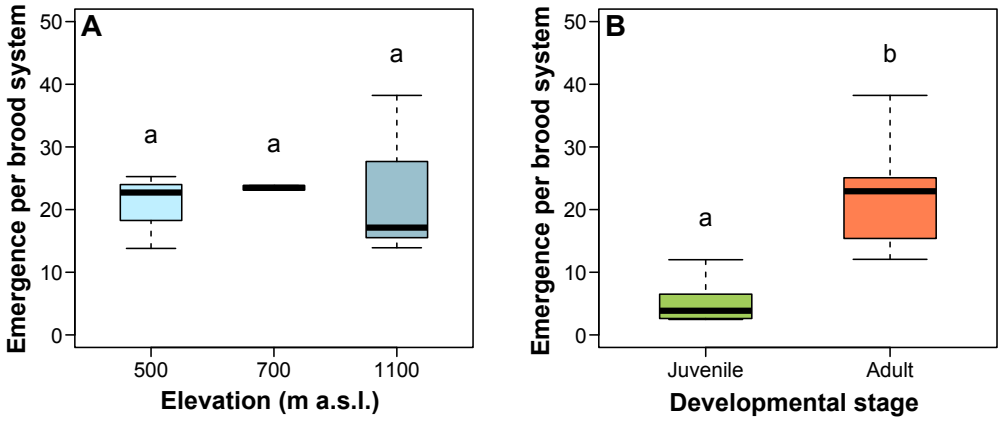


**Fig. 3.11.** Emergence per brood system after overwintering. **A.** Comparison of the three elevations of the early exposure where maturation feeding was completed before winter. **B.** Comparison of developmental stage during winter across all elevations and both exposure dates. Adult = completed maturation feeding, juvenile = larvae, pupae, callow imagines. Differing letters indicate significant differences (**A.** Approximative K-Sample Permutation test, **B.** Mann-Whitney U-test). Shown are median, upper and lower quartiles and whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box).

**Tab. 3.2.** Number of *I. typographus* individuals emerging per brood system for both exposure dates and all elevations. Shown are means with one standard error.

| Emergence per brood system | 500 m a.s.l | 700 m a.s.l | 900 m a.s.l | 1100 m a.s.l |
|---|---|---|---|---|
| early exposure | 20.6 ± 3.5 | 23.5 ± 0.4 | 23.1 ± 7.6 | 5.7 ± 1.8 |
| late exposure | 23.7 ± 3.6 | 3.1 ± 0.6 | 10.0 ± 1.6 | 2.8 ± 0.2 |

Fresh and dry masses and lipid contents (as acylester equivalents) of surviving beetles showed a decreasing trend with increasing elevation. The only exception were fresh masses of beetles from 900 m a.s.l. which were significantly higher than those of beetles from the other elevations (Approximative K-Sample Permutation test: maxT = 3.01, p = 0.01, N = 80, NDWD post-hoc test). Dry masses and lipids per dry mass of beetles at the highest elevation were significantly lower than those of beetles at the lower three elevations (all maxT > 4.52, all p < 0.0001, all N = 80, NDWD post-hoc test). In contrast, there were no significant differences of beetle sizes and achieved flight distances between elevations (all maxT < 1.55, all p > 0.41, all N = 80, Fig. 3.12).

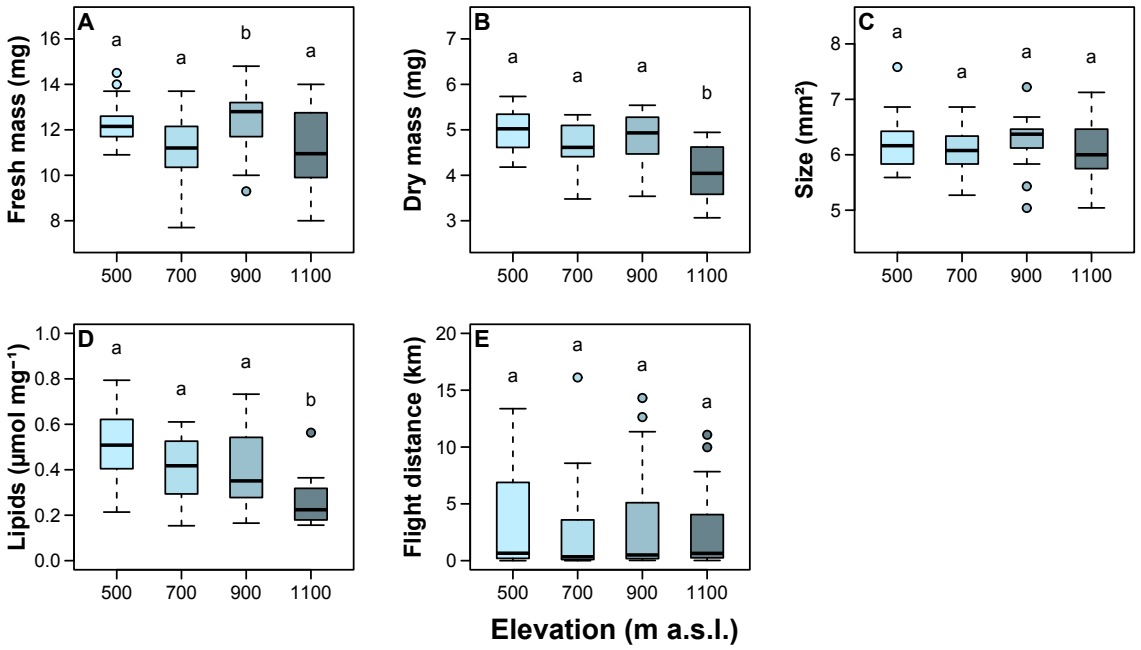


**Fig. 3.12.** Fitness parameters of overwintered beetles of the first exposure date, separated by altitudinal level: **A.** Fresh mass, **B.** dry mass, **C.** size as product of elytra length and pronotum width, **D.** lipid reserves as acylester equivalents per dry mass and **E.** flight capacity. Differing letters indicate significant differences (Approximative K-Sample Permutation test, NDWD post-hoc test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

When comparing beetles which had completed maturation feeding before winter with beetles which entered winter as pre-imaginal stages, fresh and dry masses and flight distances of the latter were significantly lower (Mann-Whitney U-Test: all $Z > 2.75$, all $p < 0.01$, all $N = 80$, Fig. 3.13a, b, e). In contrast, beetle sizes and lipids per dry mass were not significantly different (all $Z < 1.06$, all $p > 0.10$, all $N = 80$), although showing the same trend with slightly higher medians in beetles which had completed maturation feeding (Fig. 3.13c, d).

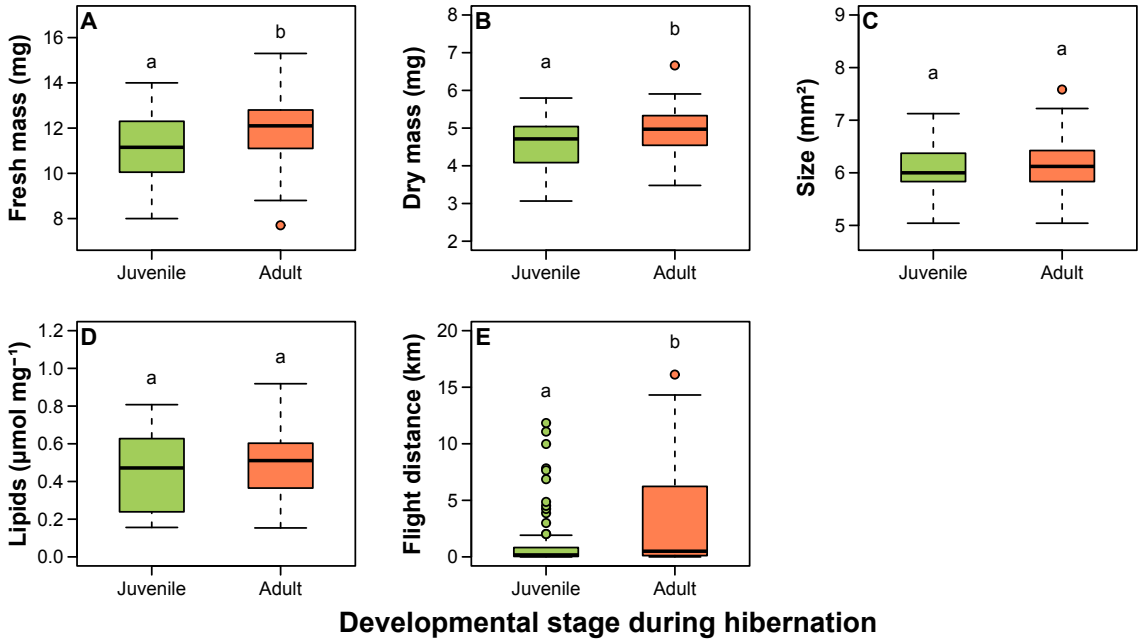


**Fig. 3.13.** Fitness parameters of overwintered beetles across both exposure dates and all altitudinal levels, separated by their developmental stage during hibernation. **A.** Fresh mass, **B.** dry mass, **C.** size as product of pronotum width and elytra length, **D.** lipids as acylester equivalents per dry mass and **E.** flight distances. Adult = completed maturation feeding, juvenile = larvae, pupae, callow imagines. Differing letters indicate significant differences (Mann-Whitney U-test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

Surviving beetles overwintering at 500 and 1100 m a.s.l. had a considerably different genetic population structure, though being statistically just non-significant (pairwise $F_{ST} = 0.0101$, exact G-test: $\chi^2 = 35.68$, $df = 24$, $p = 0.06$, $N = 94$). Observed heterozygosity ($H_o$) and mean number of alleles per locus (MNA) of emerged beetles at 500 m a.s.l. were higher than those of beetles at 1100 m a.s.l. (means ± s.e., 500 m a.s.l.: $H_o = 0.394 \pm 0.003$, MNA = 8.42 ± 1.22; 1100 m a.s.l.: $H_o = 0.360 \pm 0.003$, MNA = 7.75 ± 0.85).

### 3.3.2 Tree vs. soil overwintering: mechanism and influence on fitness

Overall, 7192 *I. typographus* individuals were caught in the 80 soil emergence traps (Fig. 3.14) and 1350 individuals emerged in the laboratory from the sampled bark between April 20th and 29th 2009. Even in soil emergence traps at trees which had already been infested in May 2008, where beetles had been expected to initiate a second generation in summer 2008, 440 beetles were caught in spring 2009 (mean ± s.e. per trap = 36.67 ± 4.55, Fig. 3.14).

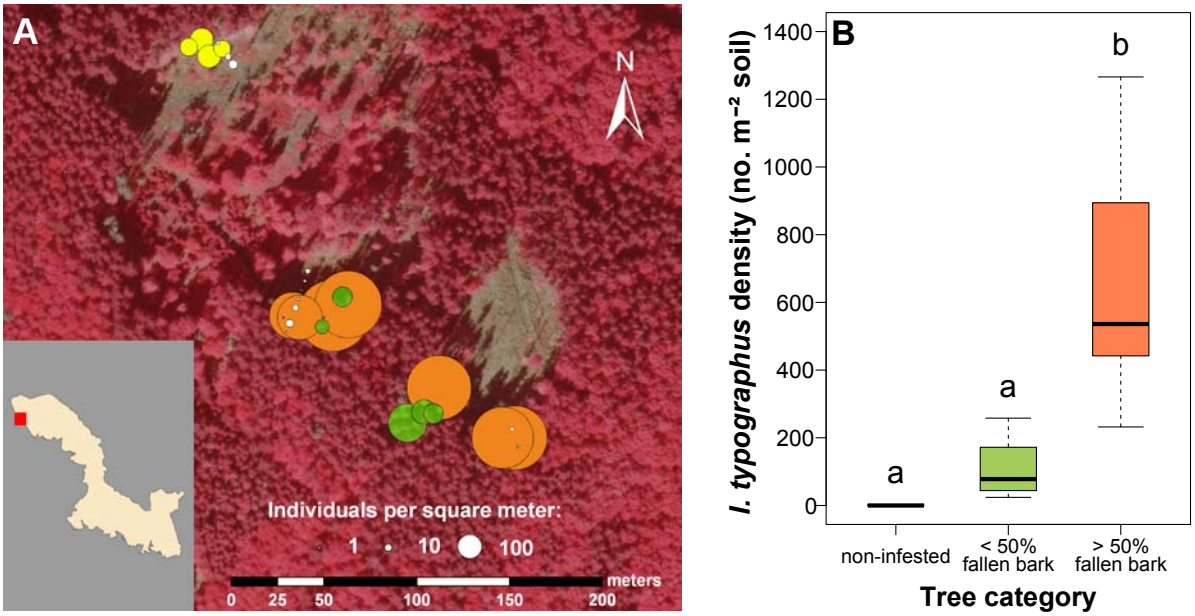


**Fig. 3.14.** Location and number of overwintered *I. typographus* caught in soil emergence traps. **A.** Size-coded (see legend) number of caught individuals per square meter soil at each tree where at least one beetle was caught. Yellow = trees infested in previous spring, orange/green = infested in previous summer with more/less than 50% fallen bark, white = gradient traps (compare Fig. 3.4). Location within the Bavarian Forest National Park is shown on the generalised map in the bottom left corner. Aerial image with kind permission by Bavarian Forest National Park Administration, Grafenau. **B.** Comparison of individual numbers per square meter between tree categories (infestation in previous summer only). Differing letters indicate significant differences (Approximative K-Sample Permutation test, NDWD post-hoc test). Shown are median, upper and lower quartiles and whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range

At the base of infested trees with more than 50% fallen bark significantly more beetles overwintered in the soil than at the base of with less than 50% fallen bark and at non-infested trees. Beetle numbers at the base of infested trees with intact bark and of non-infested trees were not significantly different (Approximative K-Sample Permutation test, NDWD post-hoc test: maxT = 4.90, $p < 0.0001$, N = 30 traps, Fig. 3.14). Moreover, the number of soil overwintering *I. typographus* individuals increased significantly with increasing quantity of fallen bark on the ground (Spearman rank correlation test: $r_S = 0.86$, $p < 0.0001$, N = 30 traps, Fig. 3.15).

Significantly more beetles emerged from the soil where bark was not removed beneath traps (paired-sample Wilcoxon signed rank test: $Z = 3.20$, $p < 0.001$, $N = 14$ trap pairs), showing that a majority of beetles was located in the fallen bark pieces and not in the soil litter (Fig. 3.16). The number of beetles decreased significantly with increasing distance from infested trees, best fitted by an inverse power law function (adjusted $R^2$: exponential model = 0.76, potential model = 0.92 with 'y = 110.58 * x^(-1.99)'; Spearman rank correlation of the distance-beetle number log-log data: $r_S = 0.91$, $p = 0.04$, $N = 5$ distances with 6 traps each). Already in a distance of 5 m almost no beetles emerged from the soil (Fig. 3.17).

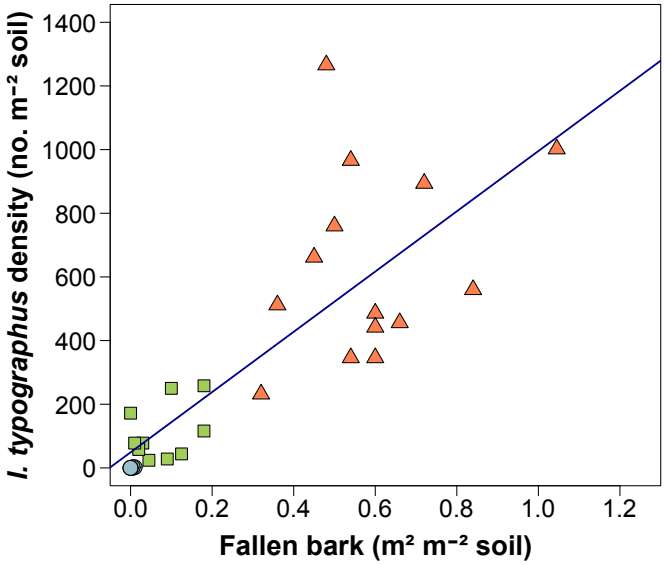


**Fig. 3.15.** Relationship between the number of soil overwintering individuals and amount of fallen bark ($r_S = 0.86$). Circles = non-infested trees, squares = infested trees with less than 50% fallen bark, triangles = infested trees with more than 50% fallen bark.

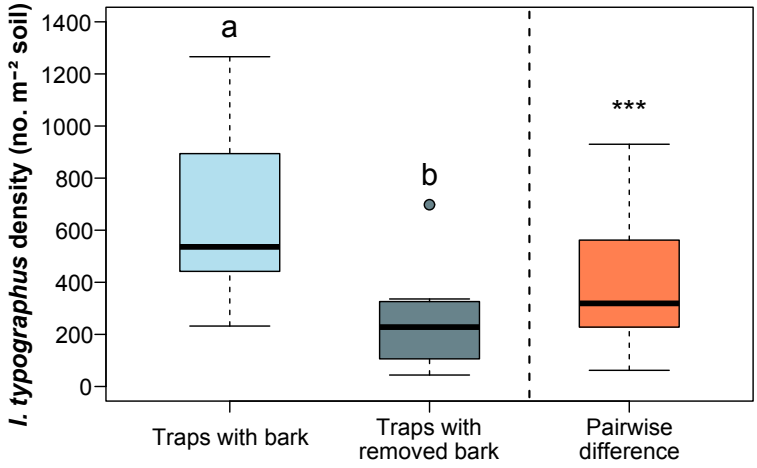


**Fig. 3.16.** Comparison of soil overwintering *I. typographus* between trap pairs with left and removed bark: soil emerging individuals per square meter when fallen bark was left below traps, when bark was removed and pairwise difference thereof. Differing letters indicate significant differences, significance level given as *** $p < 0.001$ (paired-sample Wilcoxon signed rank test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

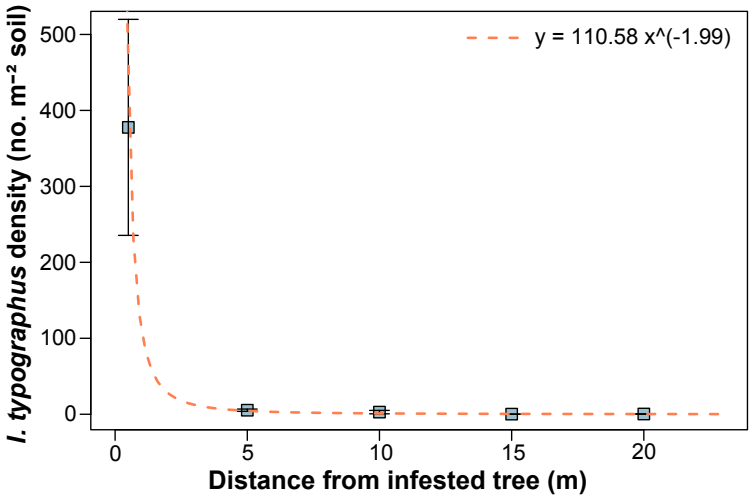


**Fig. 3.17.** Number of soil emerging *I. typographus* in relation to the distance from infested trees. Shown are means with one standard error, N = 6 traps per distance.

Sizes of beetles were not significantly different before and after winter and between the two overwintering sites (Approximative K-Sample Permutation test, NDWD post-hoc test: maxT = 1.02, p = 0.57, N = 120, Fig. 3.18c). Fresh and dry masses of beetles were significantly higher before than after winter, but they were not significantly different after winter between beetles overwintering in the soil litter and in trees (all maxT > 6.10, all p < 00.0001, all N = 150, Fig. 3.18a, b). On average, beetles lost 24% of their fresh mass and 26% of their dry mass during winter.

Beetles contained significantly more lipids per dry mass before than after winter, and beetles overwintering in the soil contained significantly more than those overwintering in trees (maxT = 8.82, p < 0.0001, N = 150, Fig. 3.18d). On average, beetles overwintering in trees lost 69% and beetles in the soil 49% of their initial lipid content. In contrast, beetles emerging from trees flew significantly longer distances than those from the soil (Mann-Whitney U-test: Z = 2.00, p = 0.04, N = 78). Generally, flight capacity of overwintering beetles was low, with 39% of individuals that did not fly at all, 46% which flew less than 100 m and only 15% flying more than 100 m with a maximum distance of 3.3 km (Fig. 3.18).

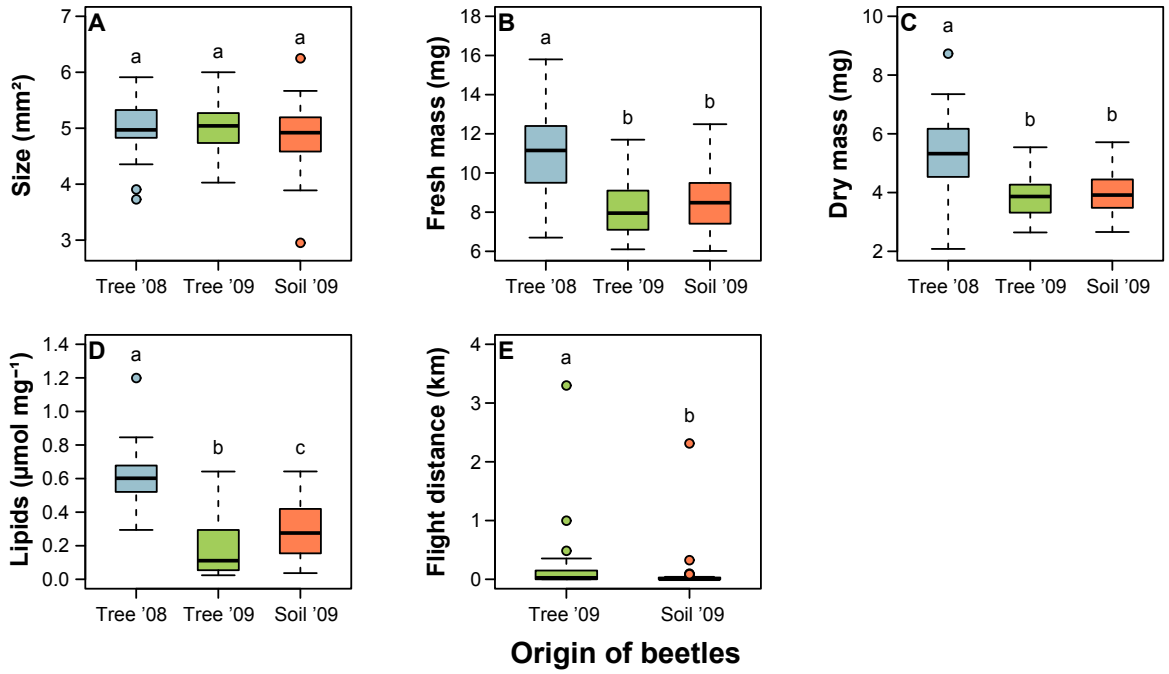


**Fig. 3.18.** Comparison of *I. typographus* fitness parameters between the different overwintering sites. **A.** Fresh mass, **B.** dry mass, **C.** size calculated as product of elytra length and pronotum width, **D.** lipids as acylester equivalents per dry mass and **E.** flight capacity of beetles sampled from trees before ('Tree '08') and after winter ('Tree '09') and of beetles sampled from the soil after winter ('Soil '09'). Differing letters indicate significant differences (**A-D**: Approximative K-Sample Permutation test, NDWD post-hoc test, **E**: Mann-Whitney U-Test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

### 3.3.3 Voltinism and time of hibernation onset

In all years, the main emergence of filial beetles occurred in the respective summer. It corresponded with the time calculated by accumulated temperature sums (Fig. 3.19). Nevertheless, a varying proportion between 11 and 49% of the respective populations remained under the bark. These individuals did not emerge before subsequent spring, despite a completed development in summer (Tab. 3.3 and Fig. 3.19).

Dry weights and sizes of filial beetles diapausing early in 2007 and 2008 were significantly smaller than those of beetles emerging for reproduction in the respective year (paired-sample Wilcoxon signed rank tests: dry weight 2007/08, plot GE: $Z = 2.91$, $p < 0.01$, size 2007/08, plot GE: $Z = 2.83$, $p < 0.01$, all $N = 27$ logs with 3634 beetles; size 2008/09, plot GE: $Z = 3.72$, $p < 0.001$, $N = 18$ traps with 1409 beetles, size 2008/09, plot GR: $Z = 3.36$, $p < 0.001$, $N = 16$ traps with 2003 beetles; Tab. 3.4 and Fig. 3.20).

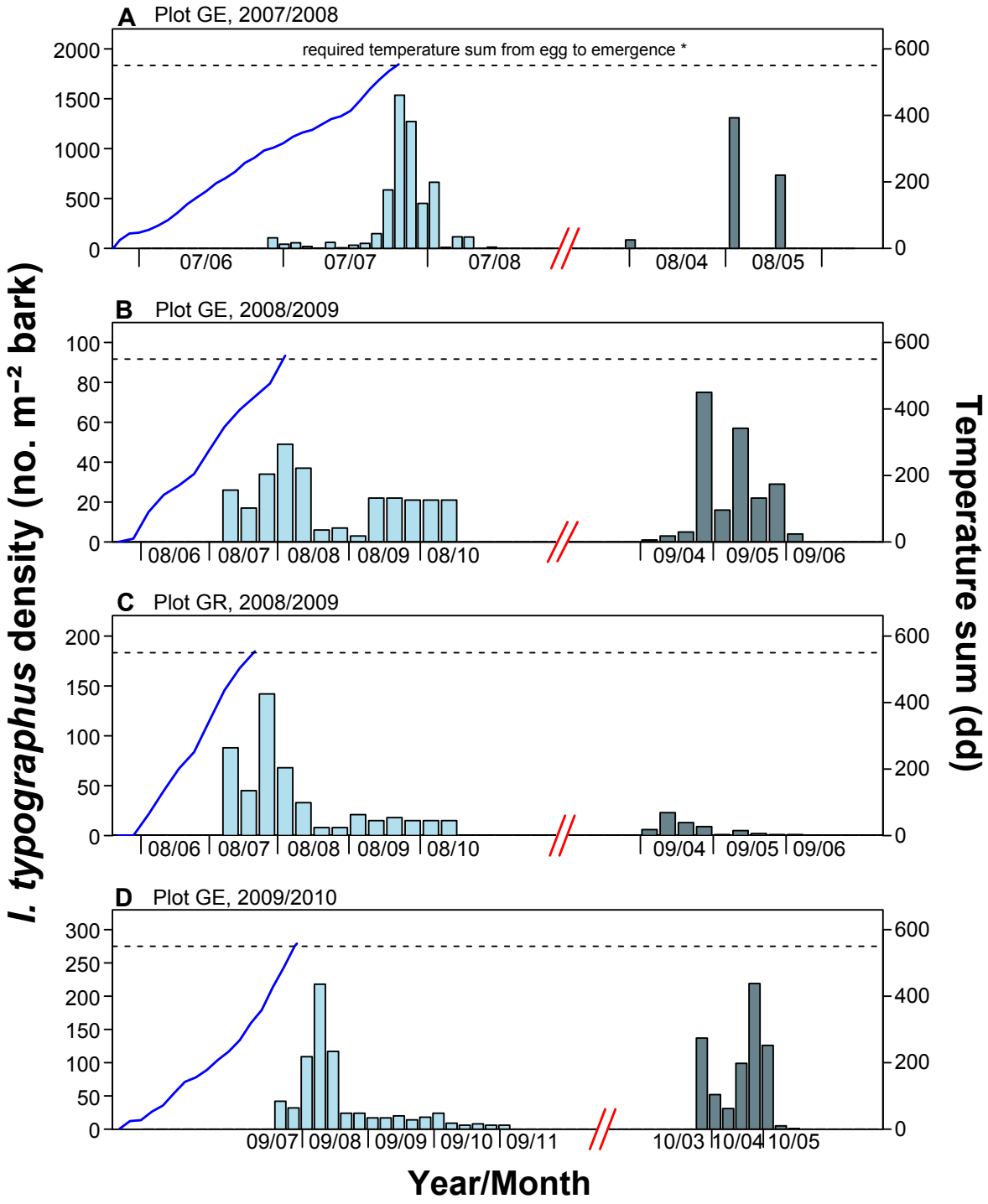


**Fig. 3.19.** Emergence pattern of first generation *I. typographus* between 2007 and 2010. **A.** Plot 'Geissberg' (GE) 2007-2008, **B.** GE 2008-2009 **C.** plot 'Grandl' (GR) 2008-2009 and **D.** GE 2009-2010. The solid curve shows the accumulated effective temperature sum over time. Its interception with the dashed line shows the point in time where development and maturation feeding of beetles is completed and complete emergence from logs was expected.

**Tab. 3.3.** Proportions of first generation individuals which entered a second reproductive cycle in a season and those which initiated hibernation in summer, even though temperature and photoperiod were in favour of an additional reproductive cycle. GE = plot 'Geissberg', GR = plot 'Grandl', N = total number of individuals sampled.

| Plot | Year of infestation | Emerged in same year | Emerged in subsequent year | N |
|---|---|---|---|---|
| GE | 2007 | 72% | 28% | 18403 |
| GE | 2008 | 57% | 43% | 1169 |
| GR | 2008 | 89% | 11% | 708 |
| GE | 2009 | 51% | 49% | 4972 |

**Tab. 3.4.** Sizes and dry weights of first generation individuals which entered a second reproductive cycle in a season and those which initiated hibernation in summer. Shown are means with standard deviation., sizes were calculated as product of pronotum width and elytra length. GE = plot 'Geissberg', GR = plot 'Grandl', $Y_1$ = year of infestation, $Y_2$ = subsequent year. Differing letters indicate significant differences between years (paired-sample Wilcoxon signed rank test, for pairwise differences see Fig. 3.20).

| Plot | $Y_1$ | $Size_{y1}$ ($mm^2$) | $Size_{y2}$ ($mm^2$) | $Weight_{y1}$ (mg) | $Weight_{y2}$ (mg) | $N_{y1}$ | $N_{y2}$ |
|---|---|---|---|---|---|---|---|
| GE | 2007 | $5.32 \pm 0.51^{a}$ | $4.99 \pm 0.56^{b}$ | $4.70 \pm 0.74^{a}$ | $4.18 \pm 0.73^{b}$ | 2459 | 1175 |
| GE | 2008 | $5.62 \pm 0.59^{a}$ | $5.26 \pm 0.64^{b}$ | - | - | 830 | 579 |
| GR | 2008 | $5.61 \pm 0.67^{a}$ | $4.93 \pm 0.64^{b}$ | - | - | 1860 | 143 |

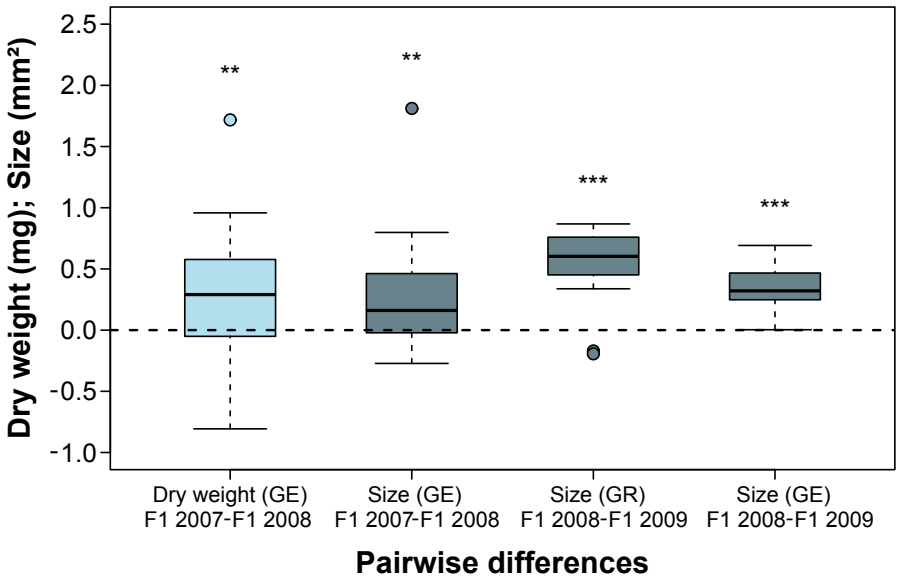


**Fig. 3.20.** Pairwise differences per trap of dry weights and sizes between first generation *I. typographus* which entered a second reproductive cycle in a season and those which initiated hibernation in summer. 'GE' = plot 'Geissberg', 'GR' = plot 'Grandl'. Significance levels are given as ** $p < 0.01$ and *** $p < 0.001$ (paired-sample Wilcoxon signed rank test, for mean values per year see Tab. 3.4). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers.

Population structure was significantly different between filial beetles which had emerged in 2009 for an additional reproductive cycle and filial beetles which stayed in the trees and emerged in 2010 (exact G-Test: all $\chi^2 > 39.43$, all df = 24, all $p < 0.03$, all N = 40, see Tab. 3.5 for pairwise $F_{ST}$-values). Observed heterozygosity and mean number of alleles per locus of beetles emerged in 2010 were significantly higher than in 2009 (paired-sample Wilcoxon signed rank test: all Z = 2.02; all p = 0.04; all N = 5 trees with 20 beetles per year and tree = 200 beetles in total, Tab. 3.5).

**Tab. 3.5.** Comparison of population structure and genetic diversity of twelve microsatellite loci between first generation *I. typographus* which entered a second reproductive cycle in a season and those which initiated hibernation in summer: pairwise $F_{ST}$-values, mean ± s.e. observed heterozygosity ($H_o$) and mean number of alleles per locus (MNA) of emerging individuals from the five studied trees in 2009 and 2010 (N = 5 trees with 20 beetles per year and tree). Significant differences in population structure ($F_{ST}$, exact G Test, all $p < 0.03$) between 2009 and 2010 are marked with an asterisk, $H_o$ and MNA were significantly higher in individuals initiating hibernation in summer (paired-sample Wilcoxon signed rank test, all p = 0.04).

| Tree no. | $F_{ST}$ (2009-10) | $H_o$ (2009) | $H_o$ (2010) | MNA (2009) | MNA (2010) |
|---|---|---|---|---|---|
| 1 | 0.0167* | 0.338 ± 0.007 | 0.425 ± 0.007 | 5.58 ± 1.25 | 6.50 ± 1.23 |
| 2 | 0.0230* | 0.321 ± 0.007 | 0.421 ± 0.007 | 5.17 ± 0.69 | 7.00 ± 1.23 |
| 3 | 0.0259* | 0.275 ± 0.006 | 0.425 ± 0.007 | 5.33 ± 0.69 | 7.08 ± 1.39 |
| 4 | 0.0415* | 0.367 ± 0.007 | 0.442 ± 0.007 | 4.50 ± 0.50 | 6.58 ± 0.87 |
| 5 | 0.0655* | 0.392 ± 0.007 | 0.433 ± 0.007 | 5.75 ± 0.79 | 6.42 ± 0.97 |

## 3.4 Discussion

The results of this study confirm that imagines of *I. typographus* are more likely to survive winter than larvae or pupae, as shown by several authors (e.g. Annila, 1969; Coeln et al., 1996; Faccoli, 2002; Schopf and Kritsch, 2010). Emergence per brood system was significantly higher when beetles had finished development and maturation feeding (Fig. 3.11). This supports the theory that larvae and pupae are at high risk of winter mortality caused by their inability to leave the moist phloem and therefore get killed by inoculative freezing. Moreover, the large fractions of dead beetles where development but not maturation feeding was completed suggests that also freshly eclosed imagines have a considerably higher mortality risk during winter than imagines which have completed maturation feeding before hibernation (Fig. 3.10). This may be based on too low lipid reserves to enrich cryoprotectants on the one hand and simultaneously maintain metabolism on the other. Furthermore, mortality of *I. typographus* during winter increased with altitudinal level (Fig. 3.10). However, minimal

temperatures were comparable between the four studied altitude levels and far above the super-cooling point of *I. typographus* (Tab. 3.1; Annila, 1969; Koštál et al., 2011). Although frost duration at 700 and 900 m a.s.l. was approximately twice as long, mortality was as low as at 500 m a.s.l. (early exposure, Fig. 3.10). Consequently, increased winter mortality was rather caused by the shorter season and thus insufficient time for development than by temperatures. At higher elevations, less thermal sum did accumulate and, as a result, beetles were not able to complete maturation feeding. This led to individuals hibernating in the more cold sensitive pre-imaginal developmental stages or as callow adults with low energy reserves. Thus, initiating hibernation early in a season might be more favourable for *I. typographus* than initiating a second generation, which would be less likely to survive under certain conditions.

Overall, fitness parameters of beetles were not significantly different after winter between elevations. Only dry masses and lipid reserves of individuals from the highest altitude level were significantly lower than those of individuals from the lower altitudes (Fig. 3.12). Lipid reserves were steadily declining with rising altitude level, which might be due to the longer time period below the freezing point. Therefore, beetles also have to invest more energy reserves for cryoprotectants (Hahn and Denlinger, 2007; 2011). These lower lipid reserves may have a negative impact on colonization success when beetles have no opportunity for a regeneration feeding, because energy is needed for detoxification of secondary host tree compounds (Bohlander and Schopf, 2000; Gatehouse, 2002; Gries et al., 1990; Raffa et al., 2005), which can increase extensively during the colonization process (Franceschi et al., 2005). Additionally, beetles at all altitudes had the same size which is determined in the larval stage, but those at higher altitudes had less time for maturation feeding which resulted in lower dry weights and lipid reserves. However, these lower energy reserves did not translate into a lower flight capacity (Fig. 3.12), which is also a crucial factor of population dynamics. Again, significant differences became obvious when considering not just altitude levels but the predominant developmental stage during winter (Fig. 3.13). At sites where beetles were able to finish maturation feeding before winter, they were significantly heavier and were able to fly significantly farther. Thus, temperature seems to influence survival success during winter not directly, but rather indirect through the duration of the time period in which temperatures are suitable for development and maturation feeding.

Surviving beetles overwintering at the lowest and those at the highest altitude had a considerably distinct genetic population structure and also showed a trend towards a higher heterozygosity at the lower altitude (Fig. 3.14). In contrast, Gast and Stock (1994) found that

the proportion of more heterozygous individuals of overwintered *I. pini* was higher than in non-overwintered ones. They argued that more heterozygous individuals are favoured under severe conditions. However, the results presented here showed that a completed development before winter has a larger influence on survivability and fitness than minimal temperatures. Thus, populations in environments like lower elevations of the temperate zone, where the time of the first frost is highly unpredictable, might need to be more flexible in terms of genetic diversity. On the contrary, at high elevations, where the season is almost never long enough to complete the development of two generations, might favour more homogeneous populations. This hypothesis is supported by the results of Gruppe (1997), who found population differentiations with the same trends on the basis of isozyme markers between lowland *I. typographus* populations and those of higher elevations.

To sum it up, in this chapter it was shown that overwintering success is considerably higher when *I. typographus* enters winter with completed maturation feeding. Larvae, pupae and probably even imagines without completed maturation feeding experience high mortality rates, which can severely influence population dynamics if the summer generation is initiated too late in the season. As a result, population structure is shifted towards imagines over winter, which may contribute to synchronize spring swarming and host attack because all individuals of a population are prepared to disperse when conditions therefore are met. Nevertheless, fitness of surviving beetles seemed to be independent of minimal temperatures and duration of cold period. Thus, in Central Europe, temperature seems to play only an indirect role. Time for completing development and maturation feeding decreases with increasing elevation and latitude. As a result, the probability of overwintering in a cold sensitive developmental stage increases, especially when a second generation is established in the same season.

At least at the two lower altitude levels of the first exposure, beetles were expected to leave the logs in autumn or early winter. It was observed that large fractions of *I. typographus* populations leave their host trees in September and October (Annila, 1969; Botterweg, 1982) and that large fractions overwinter in the soil litter across Europe (Franz, 1948a; b; Hrasovec et al., 2011; Kuhn, 1949; Quaschik, 1953; Schmidt-Vogt, 1989; Schneider-Orelli, 1947). However, in some of these studies sampling was quite selective and very high beetle numbers were found in the areas of sanitation fellings or timber deposits (e.g. Franz, 1948a; b; Schneider-Orelli, 1947), where trees get severely damaged and beetles may reach the ground passively. The very small number of beetles that emerged from the logs before winter in this

study (less than 3%, Fig. 3.10) indicated that the primary location for hibernation in *I. typographus* might be the stem. This is supported by several other studies, where only small fractions of *I. typographus* populations were found to hibernate in the soil litter (e.g. Biermann, 1977; Harding and Ravn, 1985; Onysko and Starzyk, 2011; Zumr, 1982). The latter was confirmed in the second part of this study. There, not only beetle numbers under the bark and in the soil litter were counted after winter, but moreover, beetle numbers were correlated to the degree of fallen bark of infested spruces. It was shown that the number of soil overwintering beetles was significantly higher around trees with large amounts of fallen bark (Fig. 3.16). Furthermore, beetle number in the soil significantly increased with the amount of bark on the ground (Fig. 3.17). These findings support the hypothesis that the mechanism of soil overwintering in *I. typographus* is passively mediated through bark which is falling to the ground during winter. Kuhn (1949), who reported that beetles were leaving trees between September and December, already asserted that bark pieces dropped to the ground contribute to the number of soil overwintering beetles. Due to bark decomposition during winter, these beetles may not be found within the (decomposed) bark pieces in the following spring.

The passive mechanism observed in this study gets even clearer when considering the trap pairs with left and removed bark at the same tree. When the fallen bark was removed, significantly less beetles were emerging from the soil in spring (Fig. 3.18), which shows that a significant number of soil overwintering beetles did not leave trees actively, but dropped to the ground within their brood material. Furthermore, in accordance with earlier studies, individual numbers decreased rapidly with increasing distance from infested trees (Fig. 3.19; Bender, 1948; Biermann, 1977; Botterweg, 1982; Kuhn, 1949). A possible explanation of the emergence in autumn and spring in earlier studies conducted in Germany (Bender, 1948; Kuhn, 1949; Wild, 1953) and the declining distribution around host trees may lie in the difference of bark and air temperature (Biermann, 1977). Depending on orientation and stand density, insolation can heat up the bark of infested trees far above air temperature (Annila, 1969). This phenomenon can be favoured by the missing cooling effect of xylem water transport when trees are already dead. In cases when bark temperature rises above 23°C, it can deregulate the diapauses-inducing effect of short-day photoperiods (less than 16 hours light per day) and thus stimulate beetles to emerge (Doležal and Sehnal, 2007a). When surrounding air temperatures are then below 16.5°C, which is the minimum threshold for *I. typographus* flight (Lobinger, 1994), beetles would remain in the soil litter near to their host tree.

The similar body sizes of beetles before and after winter showed that tree and soil overwintering beetles originated from one collective. Over winter, individuals decreased significantly in their fresh and dry masses and their lipid reserves, independent of hibernation site (Fig. 3.20). Lipid reserves of tree overwintering beetles were significantly lower than those of beetles overwintering in the soil litter. This might be due to lower minimal temperatures under the bark of standing trees (Annila, 1969; Bakke, 1968; Leather et al., 2008). Thus, beetles are most likely forced to produce a larger amount of cryoprotectants than under the snow cover in the soil. Additionally, insolation during the day heats up stems whereas the temperature underneath the snow cover remains constant. As a result, respiration rates of tree overwintering beetles should be higher and therefore a larger quantity of stored energy is metabolized. However, flight capacity was higher in individuals overwintering in standing trees. Thus, the overwintering site seems to have only a minor influence on spring dispersal distances. At both locations energy reserves may be depleted in a way that a regeneration feeding before spring dispersal is necessary, as indicated by several authors (Botterweg, 1982; Doležal and Sehnal, 2007a; Franz, 1948a; b; Postner, 1974). Franz (1948b) observed that beetles gather at fallen bark pieces and sun exposed tree areas for such a regeneration feeding in spring. He hypothesized that they reach such sites by walking. Comparison of the achieved flight distances to beetles before winter were omitted in this analysis, because results would be severely biased due to the degeneration of flight muscles before diapause (e.g. Doležal and Sehnal, 2007a). However, the flight distances observed in the soil as well as in trees were considerably shorter than those of laboratory beetles and conspecifics from a field summer generation (compare Fig. 3.20 and Fig. 5.1).

Despite the disadvantages of large temperature fluctuations and the risk of inoculative freezing and especially chilling injuries, overwintering in the tree might also be beneficial. At least for imagines, which usually leave the phloem to drier, outer parts of the bark, the risk of inoculative freezing is minimized (Koštál et al., 2011; Schopf and Kritsch, 2010). Already Bender (1948) and Wild (1953) stated that survival of adult beetles is considerably higher in dry parts of the bark than in the moist phloem. Furthermore, the risk of fungal infection is minimized when staying in dry conditions such as in the bark (Doležal et al., 2009). Additionally, predator diversity and abundance should be considerably higher in the soil. Finally, the disadvantage of bark temperatures fluctuating with air temperatures might turn out to be an advantage in spring. Because trees heat up long before the snow cover has melted, beetles overwintering in trees can disperse earlier than beetles underneath the snow cover. This gives their offspring an advantage in competition for nutrients and space in newly

colonized hosts, and maybe more importantly, considerably more time for completing development.

To summarize, it was shown that soil hibernation in the study area occurred passively through falling bark. As expected, energy reserves were more depleted in beetles which overwintered in trees. Nevertheless, apart from influencing energy reserves differently, both overwintering sites have different other advantages and mortality risks. The mixed sites, mediated by a passive mechanism, spread winter mortality risks within populations and thus may contribute to prevent local population breakdowns.

Finally, the third part of this study adds a surprising piece of evidence to the hypothesis that the primary overwintering location is the host tree, and additionally illustrates a sophisticated life-history strategy of *I. typographus*. In the study area, at least two generations per year were expected (Faccoli and Bernardinelli, 2011; Faccoli and Stergulc, 2006; Wermelinger et al., 2012). In contrast, the results presented here show that considerable fractions of the studied first generation individuals remained in their host trees (Fig. 3.21). Accumulated temperature sums were sufficient for a completed development and maturation feeding at the latest until the end of July in all cases. Thus, temperatures and photoperiod after completion of maturation feeding should have triggered an additional reproductive cycle in all individuals (compare Fig. 3.21 and Doležal and Sehnal, 2007a). Consequently, empty host trees were expected after summer emergence. Nevertheless, in the three years studied, between 11 and 49% of the populations remained in their host trees until following spring. Thus, not only did beetles not leave their host tree in autumn for soil hibernation in the first part of this study, but moreover, considerable parts of first generation individuals remained under the bark early in a season. When insects have to cope with resources or climate fluctuating in time, they are forced to synchronize their life-cycle to these seasonal changes (Tauber et al., 1986), which was termed ‘reaction norm’ by Schlichting and Pigliucci (1998). In insects of temperate zones, developmental and reproductive diapause are life-cycle stages in which individuals are much more cold-hardy than active ones. Therefore diapausing insects are more likely to survive during winter, and furthermore, they minimize the risk of starvation under unfavourable conditions (Leather et al., 2008; Tauber et al., 1986). Under inconstant and unpredictable environmental conditions, a mixed strategy, where the progeny partly hatches immediately when growing conditions are met and partly at a later date, is favoured (Stearns, 1976). Thus, each individual can decide to reproduce or to enter diapause until the next opportunity for reproduction and therefore choose between expressing two distinct

phenotypes (Taylor and Spalding, 1988). This phenomenon was coined ‘bet-hedging’ by Seger and Brockmann (1987). It implies a trade off between mean fitness and variance of fitness. At the cost of reduced reproductive rates and a likely higher mortality due to a longer period to be detected by parasitoids or predators, the phenotype with lower mean fitness may have a selective advantage under specific conditions (Philippi and Seger, 1989). In a stochastic environment, such benefit may be the drastically lower mortality of adults which completed maturation feeding before winter compared to eggs, larvae, pupae and callow adults, as shown in the first part of this chapter. In this context, Menu et al. (2000) demonstrated that a short-cycle phenotype of the chestnut weevil *Curculio elephas*, which displays a mixed strategy of emerging after one and two years, was not able to persist isolated as a result of a stochastic environment. In a bivoltine population of the cricket *Allonemobius socius* it has been shown that the proportion of non-diapausing eggs increased when mothers were reared under early summer conditions, whereas diapausing eggs increased under late summer conditions (Bradford and Roff, 1995). Additionally, the proportion of diapausing eggs increased with the age of the mother (Bradford and Roff, 1993; Roff and Bradford, 2000), showing that the mother may be able to influence the proportion of diapausing offspring. In the present study, photoperiod was the same for all individuals within years, since spring swarming in *I. typographus* occurs in a narrow time frame. In contrast, spring temperature or precipitation can continuously change from day to day which may have led to differing oviposition behaviour. However, this has to remain speculation in this study, since only clusters of maternal galleries and no single females were analyzed. But, according to the prediction of reduced mean fitness, weight and size of the individuals entering diapause early in a season were significantly lower than those of individuals reproducing twice in a season (Fig. 3.22).

Furthermore, genetic differentiation was demonstrated for populations with different diapause induction behaviour (e.g. Mousseau and Roff, 1989). Bradford and Roff (1995) showed that diapause propensity in *A. socius* increased along a latitudinal cline, most likely due to the difference in season length. When reared in their natural conditions, i.e. photoperiod and temperature, individuals originating from a univoltine population produced only diapausing eggs, and young females from mixed and bivoltine populations produced some eggs developing instantly in these ‘alien’ conditions. Accordingly, individuals from bivoltine populations produced only eggs developing directly when exposed to their natural conditions, and females from univoltine or mixed populations produced a proportion of diapausing eggs. They concluded that genetic variation modulates diapause propensity as a response to

selection. In this context, *I. typographus* populations of northern latitudes would correspond to univoltine populations in short seasons, Central European lowland populations to bivoltine populations where rather long seasons favour two generations. The montane populations studied here represent mixed ones in a rather stochastic environment in which plastic phenotypes prevail. Accordingly, beetles which remained in the tree for overwintering were significantly more heterozygous and had a significantly higher mean number of alleles (Fig. 3.23). Generally, higher heterozygosity is assumed to reflect higher fitness of individuals (Chapman et al., 2009). For plasticity, it was postulated in the 'overdominance model' (Scheiner, 1993) that it corresponds inversely with heterozygosity, i.e. the more heterozygous an individual, the less plastic it is. In this context, multivoltinism in *I. typographus* would be the plastic response to prolonged seasons of the more homozygous individuals. This would imply that that diapause in *I. typographus* is facultative, which recently has been proposed for the mountain pine beetle *D. ponderosae* (Lester and Irwin, 2012). However, today it is strongly believed that heterozygosity is not linked to plasticity at all (Pigliucci, 2005).
Moreover, additionally to differences in heterozygosity, significant genetic differentiation of the respective two *I. typographus* progeny sub-populations of each reproduction season was found in this study using microsatellite analysis (Tab. 3.5). This result indicates that the observed plasticity is rather 'conservative bet-hedging', which is achieved by a single phenotype that avoids winter mortality, than by 'diversified risk-spreading', achieved by phenotypic variation of a single genotype (Hopper, 1999).

In conclusion, the life-history strategy of partly establishing a second generation to enhance population growth and partly initiating hibernation early in a season to enhance winter survivability, can account substantially for population stability in an unpredictable environment. It adds to the phenomenon of mixed hibernation sites described above in spreading the risk of winter mortality and thus sustain local populations.

# Chapter 4: Lipid reserves and flight capacity on individual level

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*equal contribution

## 4.1 Introduction

The previous chapters have shown that larval developmental conditions have considerable effects on fitness parameters like size or energy reserves of *I. typographus*. But the differing fitness generally did not translate into differing dispersal capacity. However, initial lipid content of individuals tested for flight capacity remained unknown in these experiments. The most obstructive problem when studying fluctuations of lipid amount in the same insect individual with changing conditions over time is that current lipid measurement methods in entomology, like chromatography, vanillin or ferric perchlorate assays, require chemical extraction and thus killing the animal (Williams and Robertson, 2008). Therefore, only indirect measures like correlations with body mass adjusted for size (compare e.g. Williams and Robertson, 2008) or measuring reference individuals from the same population remain (see chapters 1.3.4 and 2.3). These indirect methods may be inaccurate and in many cases inappropriate (Green, 2001; Peig and Green, 2010). To solve this problem, zoology has, in recent years, adopted non-invasive methods widely used in human medicine. Anatomy of fossil amber arthropods was reconstructed and visualized using computed tomography (Dunlop et al., 2011; Pohl et al., 2010), morphology of an octopus was studied using ultrasound (Margheri et al., 2011) and nuclear magnetic resonance (NMR) was used to quantitatively measure body composition of small rodents (Nixon et al., 2010; Tinsley et al., 2004). NMR is a non-destructive and non-invasive technique to analyse and study the internal morphology of living specimen (Callaghan, 1992). In every living animal, there are many water protons whose NMR specific parameters like the proton spin density or chemical shift can be spatially resolved by magnetic resonance imaging (MRI) (Kuhn, 1990). With the advent of high magnetic field strengths and strong magnetic gradients, spatial resolution of up to one micrometer resolution is achievable (Lee et al., 2001). The first NMR microscopy images were obtained by Aguayo et al. (1986), who studied ova from the toad *Xenopus laevis* (Amphibia, Pipidae). Since then, a new dimension of investigating animals opened up and

many different species have been characterized by NMR microscopy: e.g. the development of a locust embryo (Gassner and Lohman, 1987), pH metabolism of living insects (Skibbe et al., 1995), development and metamorphosis of lepidopteran pupae (Behr et al., 2011; Goodman et al., 1995), metamorphosis of the silkworm (Mapelli et al., 1997) and the morphology of diving beetles (Wecker et al., 2002) and bees (Haddad et al., 2004; Tomanek et al., 1996). However, mainly due to high costs, only few zoological studies have incorporated NMR analyses. Given its unique properties, NMR 'could in fact be used more widely in zoology' (Ziegler et al., 2011).

One of the main features of NMR is that different chemical environments can be separated due to their different chemical shift. In addition to its non-invasive character this feature enables researchers to measure one insect individual several times and therefore to bypass the problem of indirect comparison methods. In cooperation with the Institute of Medical Engineering, Technische Universität München (Franz Schilling, Axel Haase) and the Department of Chemistry, Technische Universität München (Steffen J. Glaser), a method of visualising and quantifying lipid and water distribution in living *I. typographus* specimens was established. Thereafter, flight capacity of *I. typographus* as a function of lipid reserves was studied.

Additionally, the allocation of energy substrates was studied. In insects, energy reserves are stored mainly in the form of triacylglycerols in the fat body (Arrese and Soulages, 2010). The advantage of storing energy in the form of lipids is that it requires less space than isocalorific amounts of carbohydrates (Beenakkers, 1965). Insect flight muscles sustain the highest metabolic rates of all animal tissues, generating wing beats of several hundreds and in some species even more than a thousand per second (Candy et al., 1997). But only small amounts of energy precursors are present in the flight muscles. Thus, fuel for flight has to be provided by circulating energy rich substrates originating from the fat body (Beenakkers et al., 1984). Mainly because of their molecular weight, triacylglycerols cannot be circulated directly to the place of consumption and therefore have to be converted for transport (Kraußе-Opatz et al., 1995). Generally, there are three forms of energy utilised in insect flight muscles: diacylglycerols, trehalose or proline (Arrese and Soulages, 2010). Long range flying insects generally depend on the oxidation of free fatty acids in their flight muscles as fuel for flight. Therefore, fat body triacylglycerols are converted to diacylglycerols, catalysed by a lipase. Diacylglycerols are transported in the haemolymph associated with the insect lipoprotein lipophorin (Beenakkers et al., 1985; van der Horst et al., 2002). Based on body composition analyses of newly emerged and flown individuals, it was hypothesised that proline serves as

main fuel for *I. typographus* flight (Kraußе-Opatz et al., 1995). The action of a proline-alanine shuttle system for energy substrate allocation was first suggested by Bursell (1963). Thereafter it was shown that a proline-alanine shuttle system serves as main energy support for flight in several insect species (e.g. tsetse fly: Bursell, 1977, dung beetle: Pearson et al., 1979, Colorado potato beetle: Weeda et al., 1980, sawfly: Schopf, 1981). Thereby, proline is synthesised by additive reaction of acetyl-CoA units derived from β-oxidation of fatty acids and transamination. Proline is then circulated in the haemolymph to the flight muscles, where a partial oxidation provides energy in form of NADH. The end product of the reaction is alanine, which is transported back to the fat body where its carbon skeleton is carboxylated and serves again as acceptor of acetyl-CoA units (Bursell, 1977). To elucidate the role of proline as energy substrate for *I. typographus* flight, in the final part of this study proline and alanine contents were compared between freshly emerged individuals and individuals flown for varying durations.

## 4.2 Material and Methods

### 4.2.1 Method establishment, visualisation of anatomy and relative lipid distribution

*Experimental design*

Ten *I. typographus* individuals from the 29$^{th}$ generation of the laboratory stock of the Institute for Animal Ecology (see chapter 1.2.4) were studied. To immobilize beetles they were cooled down to approximately 2°C prior to the experiments. Before transferring the beetles to the NMR microimaging system, they were fixed mechanically in their position in a 5 mm Shigemi tube (Shigemi Inc., Allison Park, USA) by glass rods from the top and the bottom. Temperature inside the tube containing the beetle was adjusted to 2°C with a constant nitrogen flow around the glass tube. After NMR measurement beetles were killed by freezing them at -20°C. Water content of beetles was determined by calculating the difference of their fresh and dry mass. Therefore, beetles were dried at 60°C for 24 h. Lipid reserves were analysed as acylester equivalents using the photometric assay modified after Snyder and Stephens (1959), as described in chapter 1.2.4.

Furthermore, in addition to NMR spectroscopy, two beetles were examined by NMR microscopy before and after flight. For this purpose, cooled beetles were warmed up to room temperature after their initial NMR measurement. Then their flight capacity was tested using flight mills (see chapter 1.2.4) for twenty hours before a repeated NMR measurement.

*NMR methods*

NMR facilities were provided by Steffen J. Glaser (Department of Chemistry, Technische Universität München) and Axel Haase (Institute of Medical Engineering, Technische Universität München), NMR measurements were conducted by Franz Schilling (Institute of Medical Engineering, Technische Universität München).

Beetles were analyzed in a 14.1 Tesla NMR microscopy system (BrukerBioSpin GmbH, Rheinstetten, Germany) with gradient strengths up to 3 T/m. Magnetic field inhomogeneity was improved by manual shimming up to second order. 1D-proton spectra of the whole insect without any spatial encoding were acquired with a standard pulse-acquire NMR experiment within 16 averages and a repetition time of 4 s.

In addition to spectroscopy, multi-slice spin echo images with an in-plane resolution of 31 µm x 31 µm and a slice thickness of 150 µm were acquired. Repetition time was set to *TR* = 1000 ms, echo time was set to *TE* = 9.4 ms. A field-of-view (FOV) of 0.8 cm x 0.8 cm was used, the matrix had a size of 256 x 256, and overall acquisition time was $t_{acq}$ = 4 min 16 s. Optionally, fat saturation by using a 90° Gauss-pulse at 4.0 ppm offset with respect to the proton resonance (bandwidth = 3.5 ppm) was performed before image acquisition. Transverse magnetization was dephased by a spoiler gradient. Images with and without fat saturation were taken before and after tethered flight of beetles. Then, the difference of the two pictures yielded the fat distribution. Maps of fat distribution were calculated with ImageJ (Abràmoff et al., 2004). Acquisition time for the two images was 8 min 32 s. To identify the spatial distribution of fat more clearly, pictures with a transparent-zero projection (Abràmoff et al., 2004) were produced.

High-resolution 3D-imaging with an in-plane resolution of 12 µm x 12 µm and a slice thickness of 120 µm was performed to study the beetles' anatomy. Repetition time was set to *TR* = 1000 ms, echo time was set to *TE* = 7.3 ms. A FOV of 0.6 cm x 0.6 cm was used, the matrix had a size of 512 x 512, and overall acquisition time was $t_{acq}$ = 7 h 7 min.

*Statistical analysis*

NMR spectral signal intensities of fat and water of ten different beetles were correlated to water and lipid amount obtained by conventional measurements using Pearson's product-momentum correlations. All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011).

### 4.2.2 Relationship between amount of lipid reserves and flight capacity

*Experimental design*

Total lipid content of 29 freshly emerged spruce bark beetles was measured using non-invasive NMR spectroscopy as described above (chapter 4.2.1). Directly after the measurement beetles were tested in flight mills for 17 h under constant illumination at room temperature (see chapter 1.2.4 for detailed methods). After the flight experiments, beetles were immediately killed in a freezer at -20°C. Then lipid content of beetles was measured using the colorimetric assay described in chapter 1.2.4. Additionally, lipid content of 20 dead beetles was analysed again with NMR spectroscopy. In contrast to the destructive colorimetric method after Snyder and Stephens (1959), which detects only fatty acid esters, NMR spectroscopy detected the sum of fatty acid esters and free fatty acids. The highly significant correlation obtained in the pilot study between NMR lipid peak integral and lipid contents obtained by the destructive method showed that in recently emerged *I. typographus* individuals lipids were mainly present in the form of fatty acid esters (triacylglycerols) as they are stored in the fat body (Arrese and Soulages, 2010; Kraußе-Opatz et al., 1995), and that the amount of free fatty acids is negligible. This different characteristic of the two methods used was utilised to determine lipid mobilisation from the fat body and actual, total lipid consumption after flight. Thus, the difference between the amount of lipids before flight and after flight expressed as µmol acylester equivalents showed the amount of lipids which was mobilised. The difference between lipid reserves before flight and lipids after flight, both measured with NMR spectroscopy, showed the actual lipid consumption during flight. Consequently, the difference between fatty acid ester loss in the fat body (mobilisation, measured photometrically) and actual consumed fatty acids (consumption, measured by NMR) should be zero in case the complete lipids mobilised from the fat body were used up during the flight experiments.

Furthermore, beetle fresh mass and size (as product of total length, pronotum width and thorax height) were measured. Standardised heterozygosity based on twelve microsatellite loci was analysed of all tested beetles. Microsatellite analysis was conducted by Bernhard C. Stoeckle (Molecular Zoology, Department of Animal Science, Technische Universität München, see chapter 2.2 for detailed methods).

*NMR calibration*

Prior to each NMR run the lipid spectra of ten freshly emerged *I. typographus* individuals were measured. Lipid protons could be spectrally separated from water protons in the

experiments since there is a difference in resonance frequency between lipid and water protons (Schilling et al., 2012). Therefore, the NMR lipid peak integral is proportional to the total amount of lipids present. After the measurement beetles were immediately killed by freezing at -20°C. Then their lipid reserves were extracted and measured photometrically as described in chapter 1.2.4. The integrated NMR spectra were correlated with the amount of lipids obtained photometrically. Thus, a standard curve for each trial day was obtained so that NMR lipid peak integrals of beetles tested in flight mills could be converted to lipid content in µmol acylester equivalents (Pearson's product-moment correlation: all $R^2 > 0.93$, all $t > 7.39$, all $df = 8$, all $p < 0.001$, all $N = 10$; see also Schilling et al., 2012).

*Statistical analysis*

Flight distances were correlated with beetle fresh mass, size, absolute amount of lipid reserves, lipid reserves adjusted for fresh mass and size and to standardized heterozygosity (SH, Coltman et al., 1999; calculated using the R extension Rhh, Alho et al., 2010) using Spearman rank correlation tests. Additionally, lipid reserves and flight distances were correlated with lipid mobilisation from the fat body (concentration before flight - concentration after flight, photometric measurement) and consumption (concentration before flight - concentration after flight, NMR spectroscopy). Furthermore, the amount of lipids mobilised from the fat body was compared to the amount of lipids consumed for flight using a paired-sample Wilcoxon signed rank test. All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011).

### 4.2.3 The role of proline as fuel for flight

*Experimental design*

Proline and alanine concentrations of *I. typographus* of five different groups were analysed. The first group (Tab. 4.1: A) was analysed immediately after emergence from spruce logs and the other four groups after different durations of tethered flight (Tab. 4.1: B-E, see chapter 1.2.4 for detailed methods). In order to test amino acid concentration after different flight times, beetles of categories B-E were frozen in liquid nitrogen directly when in flight or in flight break.

**Tab. 4.1.** Treatments of beetles tested in flight mills with subsequent analysis of amino acid content.

| Group | Treatment |
|---|---|
| A | Immediately after emergence from spruce logs |
| B | Conservation during flight after a flight time of approximately 0.5 min |
| C | Conservation during flight after a flight time of at least 10 min |
| D | Conservation during flight break after a flight time of at least 10 min |
| E | Conservation during flight after a flight time of at least 10 min, a flight break and a flight time of approximately 0.5 min |

*Amino acid quantification*

Beetles were cut in three pieces between head and thorax and thorax and abdomen. Amino acids were extracted twice for 5 min with methanol/$H_2O$ (50%, v/v) in an ultrasonic bath at 30°C. Afterwards beetles were dried at 60°C for 24 h for dry mass determination. Methanol extracts were dried in an exsiccator over silica gel in a vacuum atmosphere for two weeks at room temperature (20-22°C). Dried residues were dissolved in 100 µl of 10% sulfosalicylic acid (AppliChem GmbH, Darmstadt, Germany), vortexed, cooled down to 4°C for 30 min and finally centrifuged (12,500 g) for 10 min at 4°C. Aliquots of the supernatant (50 µl) were adjusted to 100 µl with 2% thiodiglycol (sample buffer, Labor Service Onken, Gründau, Germany) and applied to an anion exchange column of an amino acid autoanalyser (Biotronik LC5001, Munich, Germany). Separation and quantification of the amino acids were performed by a standard protocol for elution buffers and temperature. Column effluent was continuously mixed with ninhydrin and heated for 11 min at 125°C. Chromatographically separated, ninhydrin stained amino acids were recorded photometrically in a flow cell at 550 nm for amino acids with a primary amino group (alanine) and at 440 nm for those with a secondary amino group (proline).

*Statistical analysis*

Proline-alanine ratio and absolute proline and alanine concentrations were compared between the five beetle categories using an ANOVA followed by a TukeyHSD post-hoc test. All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011).

## 4.3 Results

### 4.3.1 Method establishment, visualisation of anatomy and relative lipid distribution

*Spectroscopy*

Fat and water content of living bark beetles were investigated by conventional nuclear magnetic resonance techniques. $^{1}$H-NMR spectra of bark beetles showed a clear chemical shift separation between fat (4.7 ppm) and water (0 ppm), which arises from the different electronic environment of fat and water protons (Fig. 4.1a). The NMR peak integrals of lipids and water of ten beetles significantly correlated with the data obtained by the used destructive standard detection method (Pearson's product-moment correlation: water: $R^2 > 0.99$, $t = 66.04$, $df = 8$, $p < 0.0001$, $n = 10$, fat: $R^2 > 0.99$, $t = 19.99$, $df = 8$, $p < 0.0001$, Fig. 4.1b, c). Thus, such a set of independent measurements can serve as calibration of NMR integrals on every NMR spectrometer, relating the dimensionless NMR peak integrals to absolute standard units of lipids (µmol acylester equivalents) and water (mg) content.

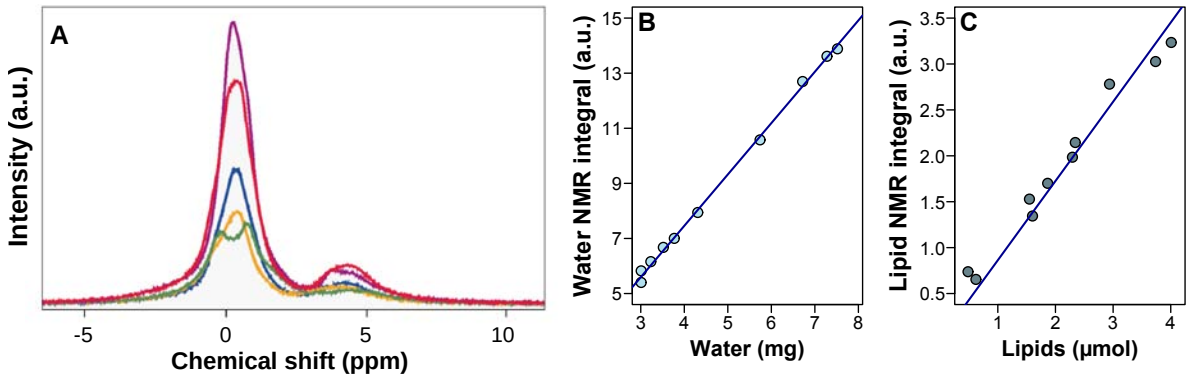


**Fig. 4.1.** Dimensionless proton NMR-spectra and their correlation to conventional water and lipid measurements. **A.** Spectra of five exemplary individuals differentiated by colours, showing clear separation between water (0 ppm) and lipid peak (4.7 ppm). Spectra were acquired using a conventional pulse-acquire 1D NMR experiment with 16 scans. **B.** NMR water response and measurement via drying of beetles ($R^2 > 0.99$). **C.** NMR lipid response and colorimetric lipid measurement as acylester equivalents ($R^2 > 0.99$). 'a.u.' = absolute units. (Figure modified from Schilling, Dworschak et al., 2012)

*Anatomy*

The anatomy of living spruce bark beetle individuals was analyzed using high-resolution spin-echo imaging. In the acquired oblique coronal slices through the beetles' body, the different body segments like the head including mouthparts, legs and wings and their respective muscles, intestinal (Baker and Estrin, 1974; Diaz et al., 2003) and genital organs (Calder,

1990) have been identified (Fig. 4.2). Susceptibility artefacts were greatly reduced by using Shigemi tubes. In addition, the Shigemi tube allowed to fix the beetle in its position and to eliminate movement artefacts caused by gradient vibrations. Cooling beetles to 2°C erased all motion artefacts during the measurement without harming them.

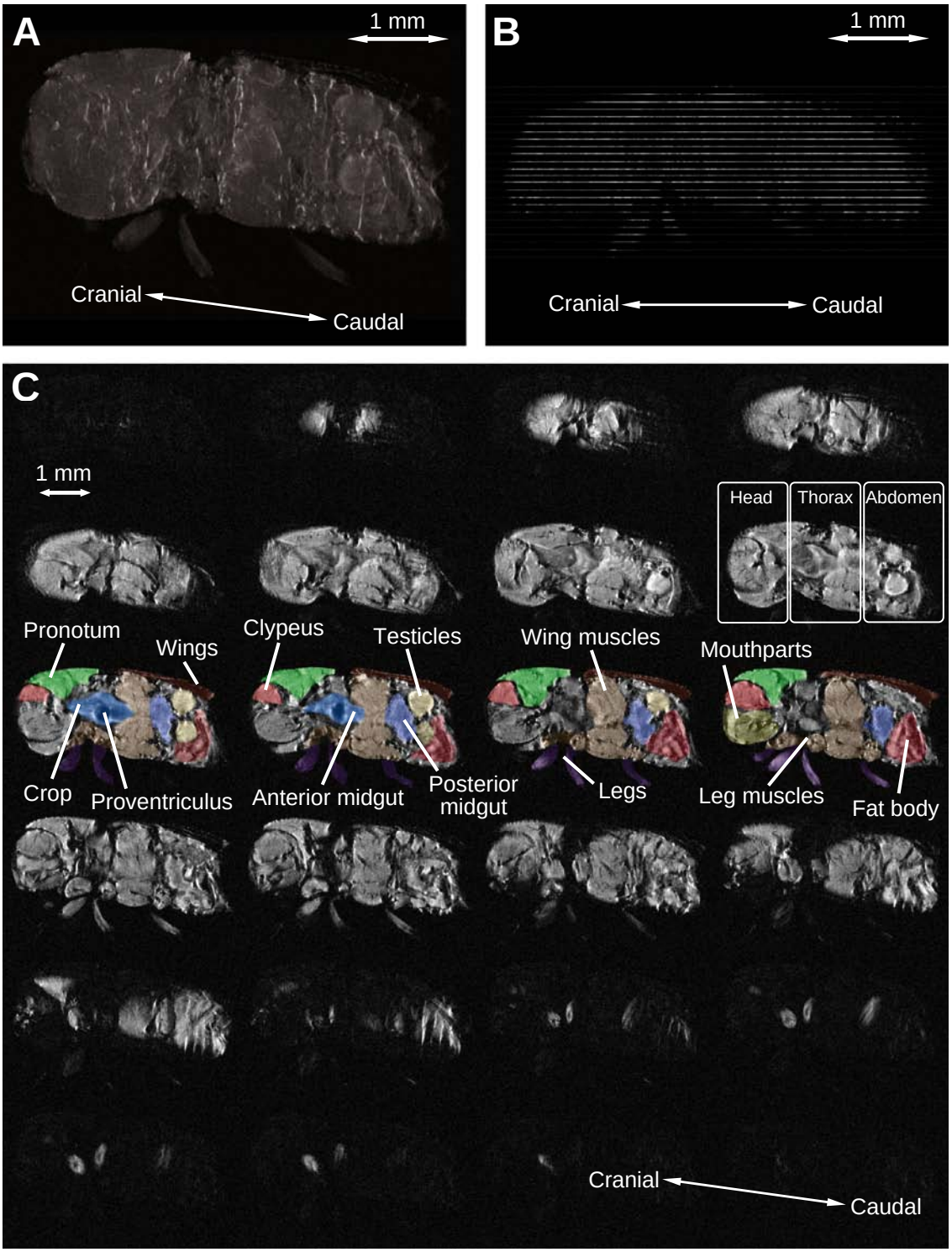


**Fig. 4.2.** Coronal magnetic resonance imaging slices of an *I. typographus* individual. **A.** Maximum-intensity-projection of all slices. **B.** Orientation of the reconstructed oblique coronal slices. **C.** Oblique coronal slices through the bark beetle with identified structures and internal organs. (Figure modified from Schilling, Dworschak et al., 2012)

*Relative fat distribution*

To image the relative spatial distribution of lipids in bark beetles, fat saturation was performed before a spin-echo imaging sequence. An image overlay of anatomy images (grey colour) with the corresponding areas of relative fat distribution (purple colour) showed lipid distribution in beetles and where lipids were depleted after flight (Fig. 4.3). In contrast to standard spectroscopic 1D-methods, signal intensity was not correlated to standard units of fat and water since signal intensities are more prone to errors. Therefore a relative spatial fat distribution was obtained. Additionally, absolute quantification of lipid and water content as well as lipid and water consumption was performed by acquiring a standard 1D proton NMR spectrum (see Tab. 4.2, Fig. 4.3 and NMR methods). One major lipid reservoir was identified in the abdomen of the beetle, most likely the diffuse fat body, and one in the centre of the body. Spatial information of the bark beetles has been combined with spectral information, which can be gained because of the spectral separation of fat and water signals. Within 8 min 32 s two high-resolution images can be acquired, allowing identification of the relative fat distribution within the beetle with regard to its internal morphology before and after flight. This method non-invasively images, in combination with spectroscopic techniques, quantifies spatial fat consumption in certain areas of interest in a specimen.

**Tab. 4.2.** Flight distances, lipid and water content of two *I. typographus* individuals measured by NMR spectroscopy before and after flight. See Fig. 4.3 for magnetic resonance imaging slices and corresponding spectra.

| | Beetle no. 1 | Beetle no. 2 |
|---|---|---|
| Lipids before flight (µmol acylester equivalents) | 1.52 | 1.69 |
| Lipids after flight (µmol acylester equivalents) | 1.14 | 1.31 |
| Lipid consumption (µmol acylester equivalents) | 0.38 | 0.38 |
| Water before flight (mg) | 3.64 | 4.92 |
| Water after flight (mg) | 2.89 | 4.13 |
| Water loss (mg) | 0.74 | 0.79 |
| Flight distance (km) | 0.34 | 13.45 |

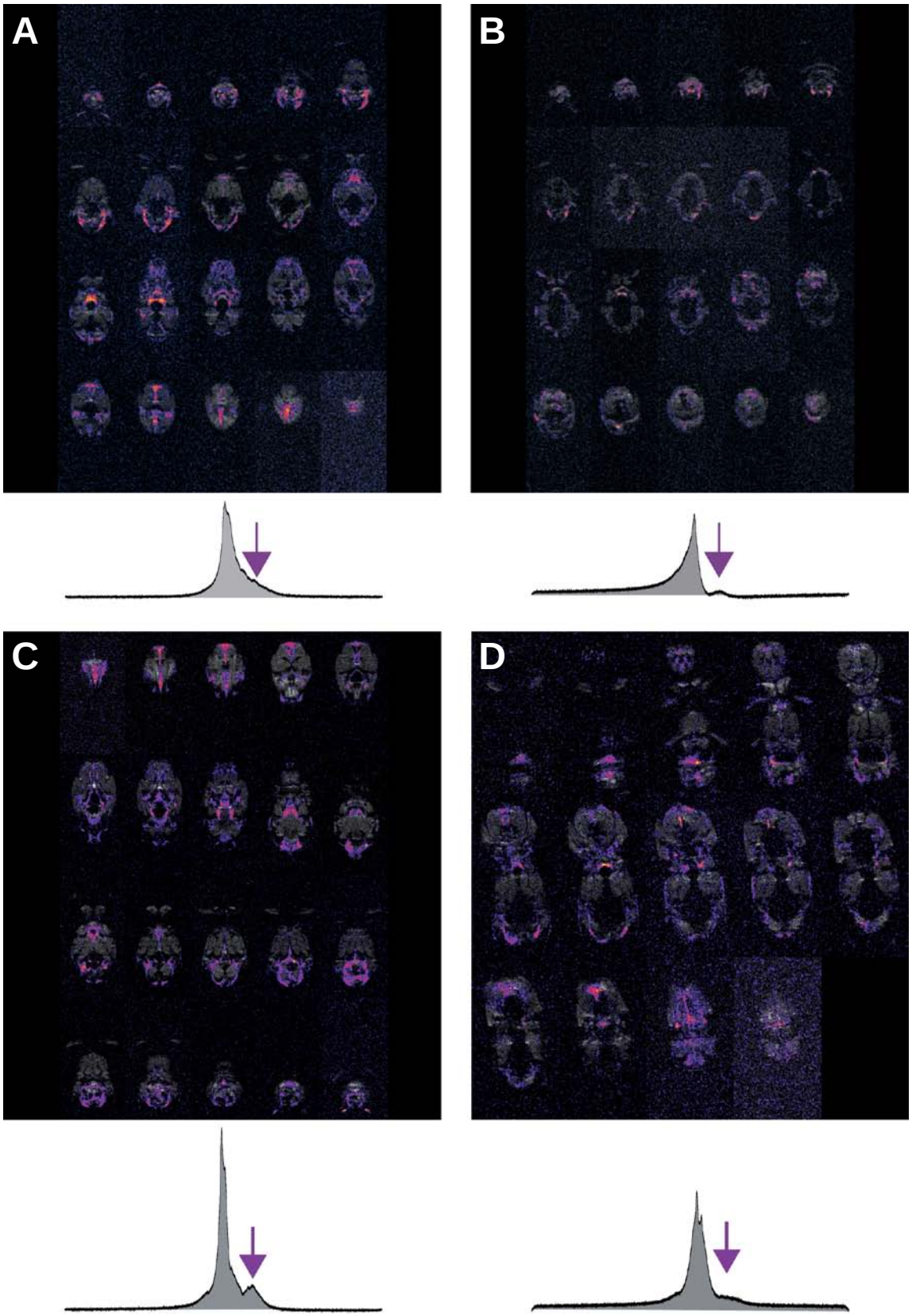


**Fig. 4.3.** Oblique sagittal MRI slices of two *I. typographus* individuals before and after flight with the corresponding NMR spectra. **A.** Beetle no. 1 before and **B.** after flight, **C.** beetle no. 2 before and **D.** after flight. Darker colour represents lower and brighter colour higher lipid concentration, darker grey tones represent lower and brighter grey tones higher water concentration. Purple arrows indicate lipid saturation pulse. Quantitative analyses of the respective NMR spectra are shown in Tab. 4.2. (Figure modified from Schilling, Dworschak et al., 2012)

### 4.3.2 Relationship between amount of lipid reserves and flight capacity

The tested beetles weighed between 12 and 13 mg. They achieved flight distances from several meters to a maximum of 14 km. Lipid reserves measured as NMR response and translated to acylester equivalents ranged between 1.8 and 3.8 µmol per beetle and between 0.15 and 0.29 µmol/mg respectively.

The covered flight distances were neither correlated with the absolute amount of lipid reserves nor with standardised heterozygosity of the respective beetles (Spearman rank correlation: all $r_S < 0.05$, all $p > 0.79$, all $N = 29$, Fig. 4.4). Furthermore, there was no relationship between flight distances and beetle fresh mass, beetle size and lipid reserves adjusted for fresh mass and size, respectively (all $r_S < 0.30$, all $p > 0.13$, all $N = 29$).

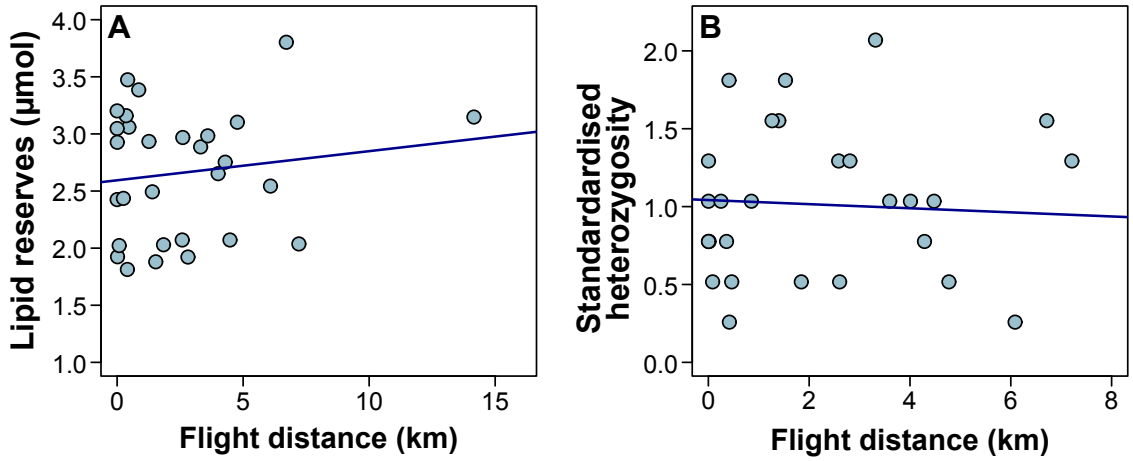


**Fig. 4.4.** Relationship between covered flight distances and lipid reserves and genetic diversity. **A.** Lipids before flight were measured as NMR response and converted to µmol acylester equivalents using a standard calibration curve (see Fig. 4.1c). Distances did not significantly increase with lipid reserves (Spearman rank correlation: $p = 0.79$). **B.** Genetic diversity expressed as standardised heterozygosity across twelve microsatellite loci.

Two factors made it possible to differentiate between lipid mobilisation and consumption. Firstly, lipids were mainly present as acylesters in freshly emerged beetles and the target of the colorimetric lipid analysis was their ester bond. Secondly, NMR spectroscopy detected free fatty acids additionally to acylesters. Thus, the difference between lipid reserves present before flight and the amount of lipids determined by the conventional photometric method after flight showed how much lipids were mobilised from the fat body (i.e. the amount of disappeared acylesters). The difference between lipid reserves before flight and the amount of lipids determined by NMR spectroscopy after flight showed how much lipids were actually consumed during flight.

Lipid mobilisation from the fat body and actual lipid consumption for flight were both independent from the amount of initial lipid reserves of beetles (mobilisation: $r_S = 0.11$, $p = 0.57$, $N = 29$; consumption: $r_S = 0.43$, $p = 0.06$, $N = 20$). Nevertheless, beetles mobilised and consumed significantly more lipids from the fat body with increasing flight distances (mobilisation: $r_S = 0.76$, $p < 0.0001$, $N = 29$, Fig. 4.5a; consumption: $r_S = 0.56$, $p = 0.01$, $N = 20$, Fig. 4.5b). However, the increase in mobilisation and consumption was disproportionately low in comparison to the increase of flight distances, shown by the slopes of the regression lines which were considerably lower than 1 (mobilisation: 'y = 0.18x + 1.14', consumption: 'y = 0.17x + 0.60'). Thus, beetles which were able to cover large distances needed less energy per given distance than those which flew only short distances. This more efficient use of lipid reserves of beetles flying long distances was even more visible when regarding the relationship between covered distance and mobilised and consumed lipid reserves adjusted for distance, which were best described by declining power law functions (mobilisation: '$y = 1.55x^{-0.88}$', adjusted $R^2$ of the potential model = 0.98, exponential model = 0.36, Spearman rank correlation of the log-log data: $r_S = -0.98$, $p < 0.0001$, $N = 29$, Fig. 4.5c; consumption: '$y = 0.88x^{-0.90}$', adjusted $R^2$ of the potential model = 0.92, exponential model = 0.38, log-log data: $r_S = -0.80$, $p < 0.0001$, $N = 20$, Fig. 4.5d).

Furthermore, the amount of lipids mobilised from the fat body, in terms of disappeared acylester groups, was significantly higher than the amount of lipids actually consumed during flight (Paired-sample Wilcoxon signed rank test: $Z = 3.81$, $p < 0.001$, $N = 20$, Fig. 4.6). The individual-wise difference of both greater than zero showed that mobilised lipids were not completely consumed (Fig. 4.6).

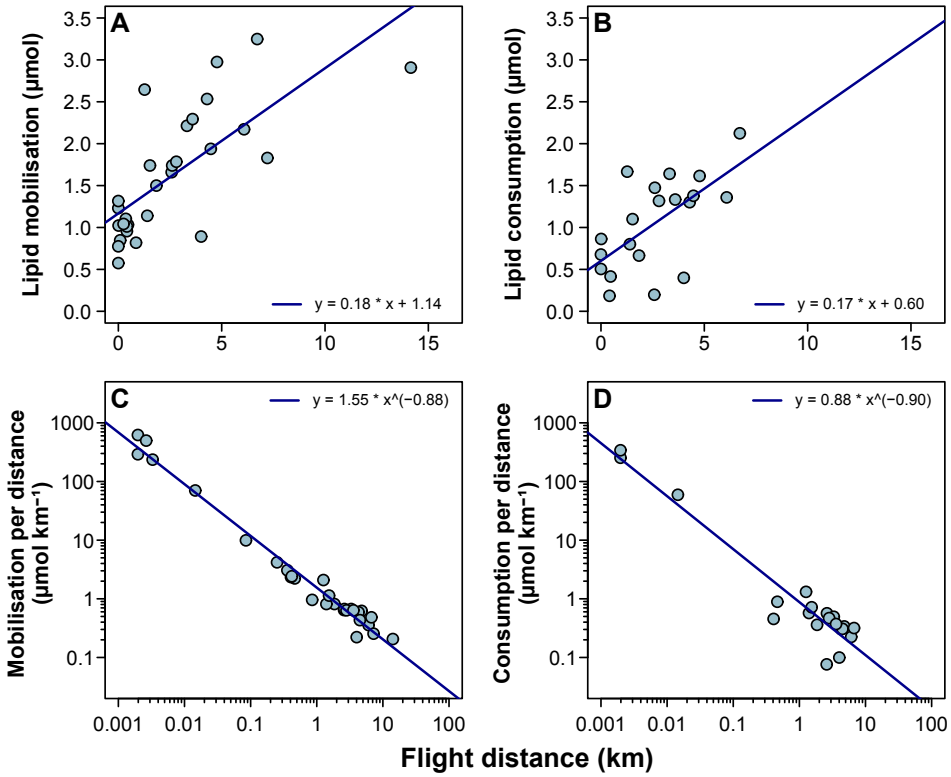


**Fig. 4.5.** Relationship between flight distance and lipid mobilisation and consumption (both expressed as acylester equivalents). **A.** Flight distances and mobilised lipid reserves from the fat body. **B.** Flight distances and consumed lipid reserves. **C.** Flight distances and mobilised lipid reserves per distance. **D.** Flight distances and consumed lipid reserves per distance. Mobilisation and consumption increased significantly with increasing flight distance, but disproportionately low (slope of the regression line smaller than 1, **A.** and **B.**). Beetles flying long distances mobilised and consumed fewer lipids per km and thus used their energy reserves more efficiently, note log scale used on both axes in **C.** and **D.**

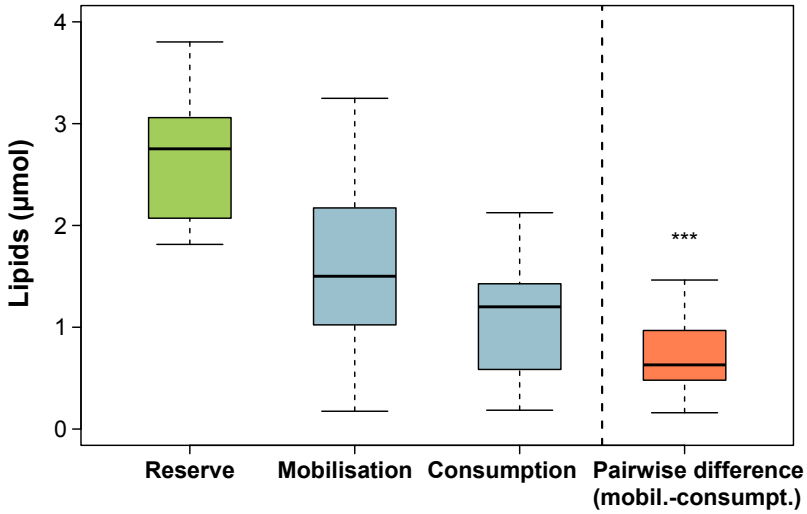


**Fig. 4.6.** Initial lipid reserves as acylester equivalents, lipid mobilisation and consumption during flight, and individual-wise differences of the two latter. Mobilisation from the fat body was significantly higher than the amount of consumed lipids (pairwise difference > 0, paired-sample Wilcoxon signed rank test: significance level given as *** $p < 0.001$). Shown are median, upper and lower quartiles and whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box).

### 4.3.3 The role of proline as fuel for flight

As expected, the proline-alanine ratio of freshly emerged beetles (category A) was significantly higher than the ratio of beetles which flew half a minute (B), and that was significantly higher than that of beetles flying 10 min and beetles in a flight break (C, D). But, contrary to the expectation, proline-alanine ratio stayed on that lower level in beetles which rested after a flight interval (E) (ANOVA: $F = 25.69$, $df = 4$, $p < 0.001$, $N = 50$, TukeyHSD post-hoc test, Fig. 4.7a). Accordingly, the proline concentration per dry mass was significantly lower in groups with longer flight and proline was not regenerated in a flight break (ANOVA: $F = 14.83$, $df = 4$, $p < 0.001$, $N = 50$, TukeyHSD post-hoc test, Fig. 4.7b). On the contrary, alanine concentration per dry mass increased only slightly and was not significantly different between the tested beetle categories (ANOVA: $F = 1.32$, $df = 4$, $p = 0.28$, $N = 50$, Fig. 4.7b).

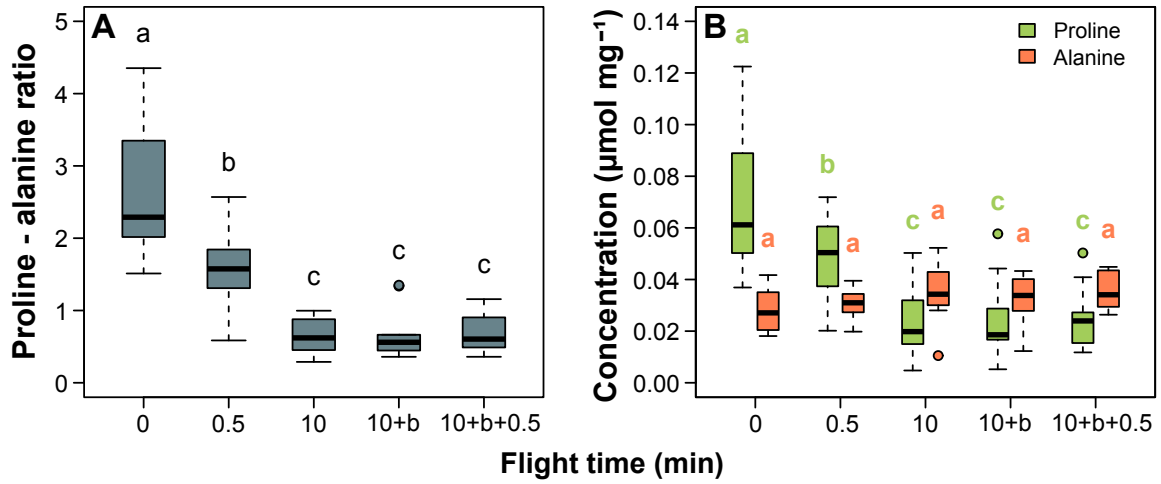


**Fig. 4.7.** Proline and alanine contents of beetles flown for different time intervals, 'b' = flight break. **A.** Comparison of proline-alanine-ratio and **B.** of absolute proline and alanine contents. Differing letters indicate significant differences (ANOVA, TukeyHSD post-hoc test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than

## 4.4 Discussion

The anatomy of living spruce bark beetle individuals was analyzed using high-resolution spin-echo imaging. With this method different body segments, like the head including mouthparts, legs and wings and their respective muscles and organs, intestinal and genital organs could be identified (Fig. 4.2). Movement artefacts were eliminated by fixing beetles in Shigemi tubes and cooling them to 2°C, without harming them. Moreover, spatial information of relative fat

distribution within individuals could be combined with spectral information (Fig. 4.3). Spectral information could be gained because of the spectral separation of fat and water signals. Thus, it is conceivable that in the future it will be possible to quantify lipid consumption or allocation of certain fatty acids in specific areas of interest over time in living insects.

Most important for the question of flight capacity as function of lipid reserves, it was shown that NMR spectroscopy can be used to non-invasively quantify lipid content and consumption of small, living insects in vivo. In contrast to conventional destructive methods, which require killing and chemical extraction of individuals (Schilling et al., 2012; Williams and Robertson, 2008), this method opened up the opportunity to directly measure flight capacity as a function of the amount of stored and consumed lipids in the same insect individual. Thus, indirect comparison methods, i.e. measuring lipid contents of reference individuals from the same population, were bypassed. Flight capacity in *I. typographus* was not correlated to the amount of stored energy reserves in form of lipids (Fig. 4.4). The missing relationship also held true when lipid reserves were adjusted for fresh mass or body size. At least in the range of lipid reserves studied here, this clearly contradicts the hypothesis that the amount of lipid reserves is directly correlated to flight capacity in *I. typographus* (e.g. Gries, 1985). Flight capacity was also not correlated to heterozygosity of beetles (see chapter 2.4 for a detailed discussion). Moreover, even though more lipids were mobilised and consumed by beetles with longer flight distances, this increase was disproportionally low in comparison to the increase in flight distance (Fig. 4.5). This result suggests that different individuals might be variable in their efficiency in transducing energy reserves into flight capacity. Reasons for a variable efficiency could be manifold. It is supposed that *I. typographus* relies on proline to fuel flight activity where proline is generated from fatty acids in the fat body, circulated to the flight muscle and alanine is transported back to the fat body (Kraußе-Opatz et al., 1995). It might be that availability of enzymes involved in the proline-alanine shuttle system are limiting and thus interrupt energy support (for the complete pathway with a full list of involved enzymes see Candy et al., 1997, Fig. 3). In the tsetse fly *Glossina morsitans* it has been shown that the rate of proline re-synthesis in the fat body is too low to keep up with proline oxidation in the flight muscle. As a result, alanine concentration in the haemolymph increases and that of proline decreases, which prevents long range flights. Proline could be replenished after some time of rest (Bursell, 1977). The results presented in this study regarding the proline and alanine concentrations after different durations of tethered flight do not support the hypothesis that proline solely fuels *I. typographus* flight. The proline-alanine ratio decreased with

increasing flight time as expected when proline is consumed and thereby alanine is enriched. However, during flight breaks the ratio stayed on the same low level and did not increase again (Fig. 4.7). This contradicts the fact that proline is replenished through addition of acetyl-CoA to alanine and transamination. When considering absolute contents of proline and alanine separately, this result was strengthened. While proline concentrations decreased by two thirds after ten minutes of flight activity and were not regenerated anymore, alanine concentrations were constant across all flight durations. This result is in accordance with studies which showed that in some insects proline is completely oxidised and its consumption does not lead to an equimolar synthesis of alanine (e.g. Hansford and Johnson, 1975; Subramanian and Varadaraj, 1985). However, it is in contrast to the findings of Kraußе-Opatz et al. (1995) who found a higher alanine concentration in landing beetles on spruces than in beetles emerging from host trees, although in one case alanine concentrations in emerging beetles was as high as in landing ones. However, history of landing beetles remained unclear in this study, whereas in the study presented here exact flight duration of each individual beetle was established. Considering the decreasing proline concentration with flight time and the constant alanine concentration, it is more likely that proline serves only as initial fuel in *I. typographus* which later in flight is replaced by diacylglycerols. It is not unusual that insect species rely on more than one energy substrate for flight (Candy et al., 1997). The prime examples are locusts which shift from carbohydrates used for initial flight to lipids mobilised from the fat body during prolonged flight (e.g. Jutsum and Goldsworthy, 1976; Wegener et al., 1986). Similarly, in several species proline serves only as minor or supplementing fuel which is then replaced by either carbohydrates or lipids (blowfly: Sacktor and Wormser-Shavit, 1966, flesh fly: Olembo and Pearson, 1982, blister beetle: Auerswald and Gäde, 1995, African fruit beetle: Auerswald et al., 1998). This might also explain the proportion of the large number of short range fliers observed in this study, which might rely on proline as sole fuel for flight, and the relatively small number of long range fliers, which might be able to switch to oxidation of free fatty acids in their flight muscles. A further explanation for the differing efficiency of energy reserve usage could be that some individuals are switching to gluceoneogenesis from glycerols after their proline reserve is exhausted. This would be less efficient than the direct usage of diacylglycerols as flight substrate. It was demonstrated that insects are capable of gluceoneogenesis from several non-carbohydrate precursors, such as glycerols, amino acids or lactate (e.g. cockroach: Storey and Bailey, 1978, mealworm: Gourdoux et al., 1983, tobacco hornworm: Thompson and Lee, 1993, locust: Candy et al., 1976).

# Chapter 5: Lipid reserves and flight capacity on population level

## 5.1 Introduction

In the previous chapters it was already shown that differing conditions during larval development influence physiological and morphological fitness traits of *I. typographus* progeny. However, it was also shown that the impact of studied developmental conditions on flight capacity was only marginal. Moreover, on an individual level it was demonstrated that flight capacity is not predictable by the amount of energy reserves. Knowledge about dispersal behaviour is the basis for the understanding of spatial distribution and gene flow between populations (Boiteau et al., 2003). Beside the reaction to semiochemicals, flight capacity is a main pillar of determining dispersal behaviour in bark beetles. Systematic knowledge about dispersal capacity in *I. typographus* and bark beetles in general is scarce (Preisler et al., 2012; Robertson et al., 2007). It is not only important as one possible driver of population dynamics, but also is a prerequisite for building simulation models of bark beetle population dynamics and thus carries essential information for the assessment of appropriate management strategies. Therefore, flight capacities in *I. typographus* are compared between populations in a meta-analysis of the results presented in the previous chapters.

## 5.2 Material and Methods

*Experimental design*

In total 1020 *I. typographus* individuals from seven (meta-) populations were compared, thereof 522 for lipid and 498 for maximal flight distance analysis. Beetles from the first five collectives (A-E, Tab. 5.1) originated from the laboratory stock of the Institute for Animal Ecology (see chapter 1.2.4) between the 6th and 33th generation. Under the assumption to generate populations with different levels of energy reserves, these collectives were reared with different population densities (A = beetles of the experiment described in chapter 2.2, D = beetles of the experiment described in chapter 4.2.2, and E), one was starved between one and four days (C = beetles of the experiment described in chapter 1.2.4) and one was placed outdoors during winter (B = beetles of the experiment described in chapter 3.2.1). Collectives six and seven (F-G) were taken from an outdoor population in the Bavarian Forest National Park, Germany (‘Grandl’, 49°5'6.37" N 13°12'46.73" E). Collective (F) consisted of parental

beetles which re-emerged in early summer to initiate a sister brood and collective (G) consisted of first generation beetles emerging in summer to initiate a second generation, both in 2008. Between 30 and 192 individuals of each collective were killed by freezing immediately after their emergence (see Tab. 5.1 for detailed information). These individuals were used to determine the distribution of lipid reserves within each collective. Furthermore, between 30 and 158 individuals were used to determine distributions of maximal flight ranges within the collectives (Tab. 5.1). Lipid reserves were analysed using the ferric perchlorate assay modified after Snyder and Stephens (1959) and maximal flight ranges were tested in the laboratory using automated flight mills (see chapter 1.2.4 for detailed methods).

*Statistical analysis*

In order to compare fat reserves and maximal flight distances between *I. typographus* collectives an Approximative K-Sample Permutation test followed by a Nemenyi-Damico-Wolfe-Dunn (NDWD) post-hoc test was performed (Hollander and Wolfe, 1999). Percentages of maximal flight distances were calculated for each collective in 500 m steps. The mean percentage with standard error was calculated for each 500 m step across all tested beetle collectives. Furthermore, to achieve better comparability between collectives, individual flight distances within each collective were related to the maximal flight distance of the respective collective, resulting in relative flight distances ranging between zero and one. Relative flight distance was calculated as

‘$d_{rel} = d_i / d_{max}$’,

with ‘$d_i$’ as the flight distance of the focus beetle and ‘$d_{max}$’ as the maximal flight distance within the focus beetle’s collective. Distribution of relative flight capacity within each collective was calculated by grouping $d_{rel}$ in steps of one tenth. Distributions of relative flight capacity were compared between collectives using two-sample Kolmogorov-Smirnov tests, corrected for False Discovery Rate in multiple comparisons after Benjamini and Hochberg (Benjamini and Hochberg, 1995). All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011).

**Tab. 5.1.** Summary of the seven *I. typographus* collectives of which flight capacity distributions were compared: origin, treatment and number of analyzed individuals used for determination of lipid reserve ('$N_{lipid}$') and flight capacity distributions ('$N_{flight}$') within each collective.

| Collective | Origin | Treatment | $N_{lipid}$ | $N_{flight}$ |
|---|---|---|---|---|
| A | lab, 6th generation (chapter 2.2) | population density 50-500 brood systems per square meter bark (pooled) | 192 | 72 |
| B | lab, 9th generation (chapter 3.2.1) | placed outdoors over winter (first exposure, pooled) | 80 | 79 |
| C | lab, 22nd generation (chapter 1.2.4) | starved between one and four days (pooled) | 100 | 70 |
| D | lab, 29th generation (chapter 4.2.2) | population density 20-50 brood systems per square meter bark | 40 | 29 |
| E | lab, 33rd generation | population density 50-300 brood systems per square meter bark | - | 158 |
| F | Bavarian Forest National Park | parental beetles emerging to initiate a sister brood in 2008 | 30 | 40 |
| G | Bavarian Forest National Park | first generation beetles emerging to initiate a summer generation in 2008 | 80 | 50 |

## 5.3 Results

Different manipulations of *I. typographus* collectives resulted in significantly different fat reserves between them (Approximative K-Sample Permutation test: maxT = 11.47, $p < 0.0001$, N = 522). First generation beetles originating from the National Park Bavarian Forest (G) and beetles from the laboratory stock reared with the lowest population density (D) had the largest fat reserves, whereas beetles from the highest population density variation (A) and beetles placed outdoors during winter (B) had the lowest reserves (NDWD post-hoc test, Fig. 5.1a). Flight distances also differed significantly between the tested *I. typographus* collectives (maxT = 6.50, $p < 0.0001$, N = 499). In contrast to their high fat reserves, beetles of collectives (G) and (D) showed rather short flight distances, whereas beetles of collective (A), which had the lowest fat reserves, achieved the farthest flight distances (NDWD post-hoc test, Fig. 5.1b).

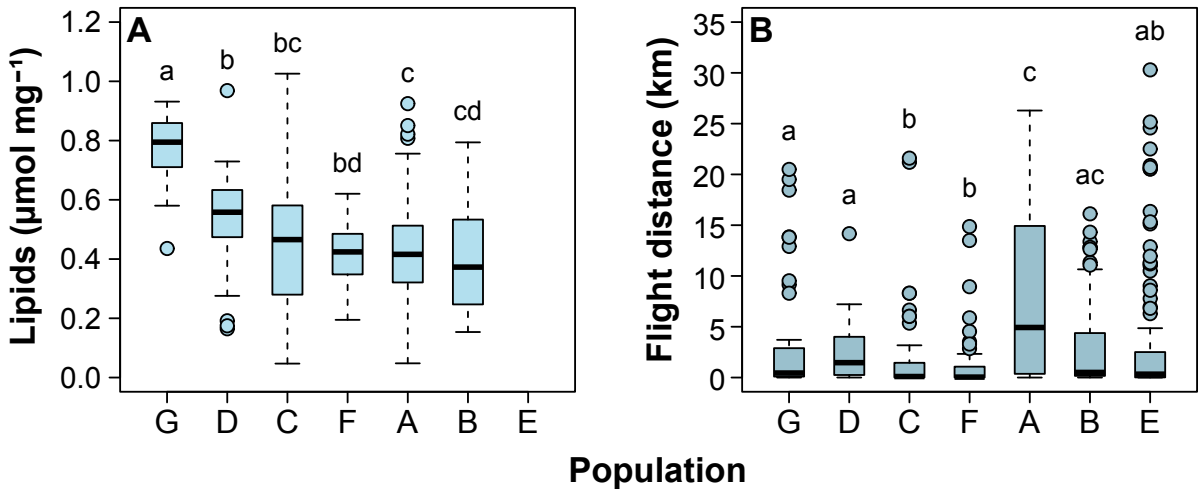


**Fig. 5.1.** Comparison of lipid reserves and flight capacity between the seven *I. typographus* collectives studied. **A.** Lipid reserves as acylester equivalents per dry mass and **B.** flight distances. Differing letters indicate significant differences (Approximative K-Sample Permutation test, NDWD post-hoc test). Shown are median, upper and lower quartiles, whiskers (which extend to the most extreme data point that is not more than 1.5 times of the inter-quartile range from the box) and outliers. See Tab. 5.1 for explanations of abbreviations and sample sizes.

Approximately half of the tested beetles (51.50 ± 5.73%, mean ± s.e.) showed a maximal flight capacity of 500 m or less (Fig. 5.2). 0.09 ± 0.03% thereof were non-fliers, which is a relatively low percentage compared to values reported in the literature of up to 30% for Scolytinae (discussed in Williams and Robertson, 2008). The distribution of flight distances was strongly shifted towards shorter ones. The main difference between collectives was in the length of the tail, accounted for by the small percentage of beetles which were able to cover extremely long distances (compare Fig. 5.1b and Fig. 5.2). The mean flight distance distribution across all seven collectives was best fitted by an inverse power law function (‘y = 9.19 * x^(-1.17)‘, adjusted $R^2$: potential model = 0.80, exponential model = 0.63).

The distribution of flight distances relative to the mean distance of the respective beetle collectives (‘$d_{rel}$’) showed this result even clearer. 64.94 ± 5.76% (mean ± s.e.) of beetles accomplished only a tenth of the relative flight distance of a given collective (Fig. 5.3). As for absolute distances, the mean distribution across all seven collectives was best fitted by an inverse power law function (‘y = 1.44 * x^(-1.40)‘, adjusted $R^2$: potential model = 0.82, exponential model = 0.57). Distributions of relative flight distances were not significantly different between the seven collectives (two-sample Kolmogorov-Smirnov tests, corrected for False Discovery Rate in multiple testing after Benjamini and Hochberg, 1995). Before correction for multiple testing, only distributions of collectives A and E were significantly different ($D = 0.7$, $p = 0.01$; all other $D < 0.5$, all $p > 0.16$), where the distribution of E was the one exhibiting the steepest decline after one tenth of relative flight distances (Fig. 5.3).

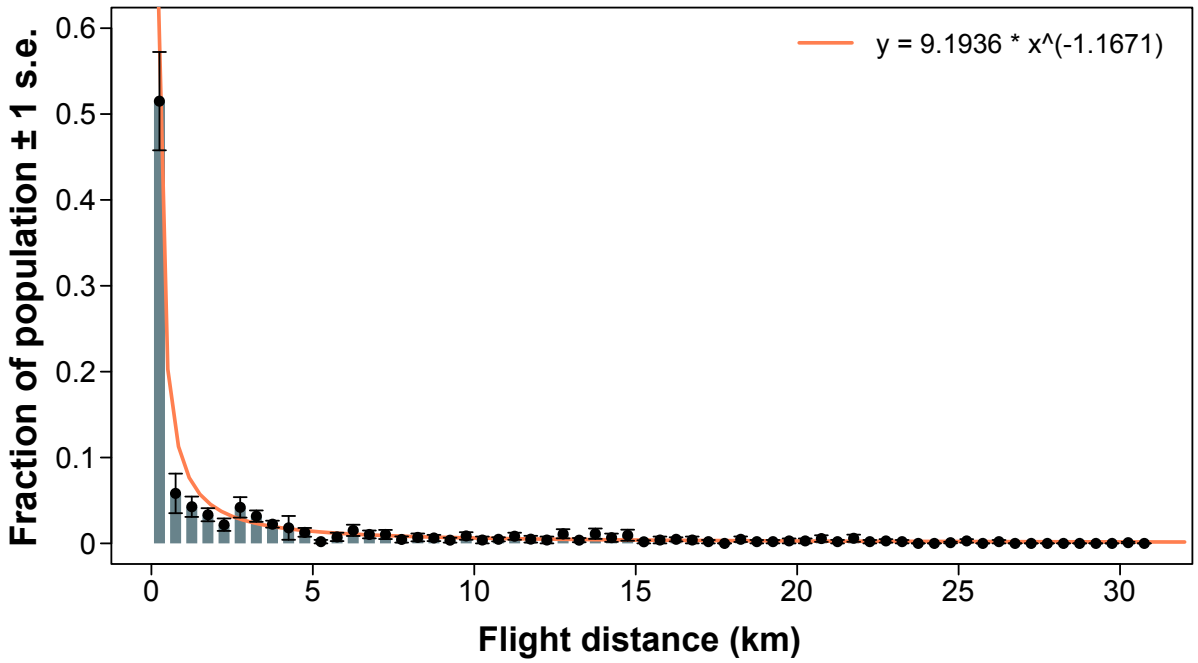


**Fig. 5.2.** Mean flight capacity across the seven studied *I. typographus* collectives in 500 m steps. Shown are means with one standard error (s.e.). The distribution was best fitted by an inverse power law function, indicated by the solid curve.

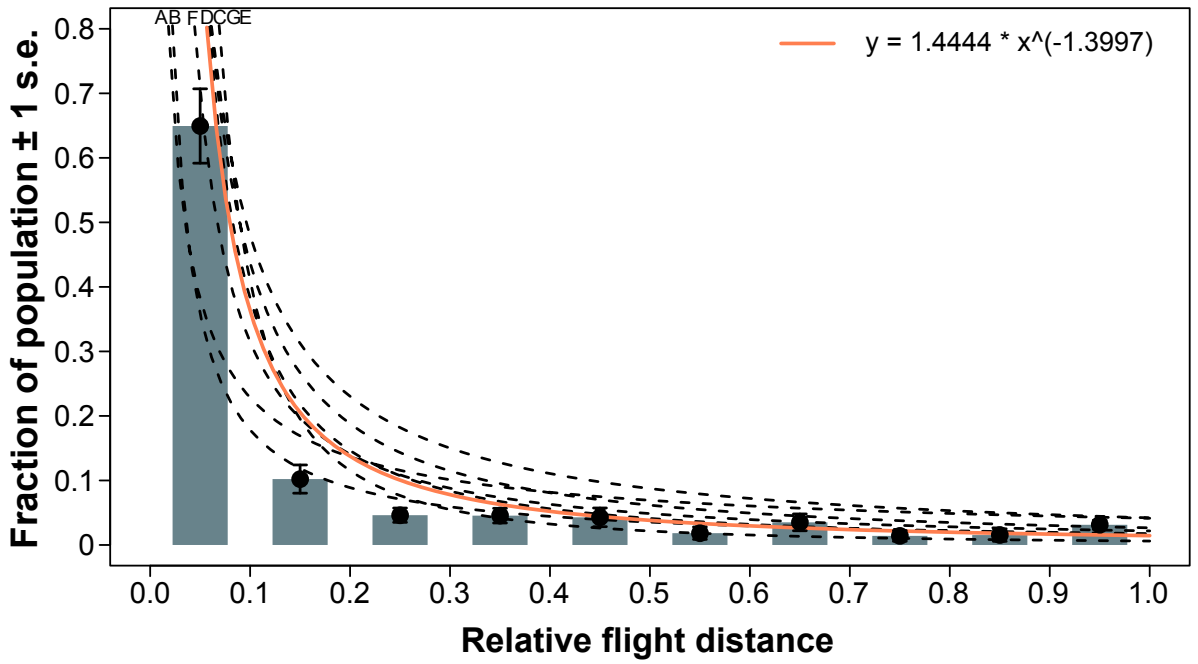


**Fig. 5.3.** Mean relative flight capacity in 10% steps across the seven studied *I. typographus* collectives. To obtain relative distances, each individual distance was divided by the maximal flight capacity within the respective collective. Shown are means with one standard error (s.e.). The mean distribution is best fitted by an inverse power law function, indicated by the solid curve. The functions for the seven single collectives are indicated by the dashed curves, letters (A-G, see Tab. 5.1) indicate the order of the curves of the respective collectives.

## 5.4 Discussion

This meta-analysis confirms the results presented in the previous chapters of this study. Although medians of flight capacity were significantly different across the seven tested populations, they did not correspond analogously to medians of lipid reserves (Fig. 5.1). Moreover, the distributions of achieved flight distances were comparable between populations. About half of the individuals within each population achieved flight distances of only 500 m or less. All distributions were strongly skewed to shorter distances and best described by a negative power law function (Fig. 5.2). When relating individual flight capacities to the maximal capacity within each population it became clear that more than 60% of a population can achieve only one tenth of the maximal capacity (Fig. 5.3). High flight capacity enables to forage for suitable host trees in an environment where the focal host species is rather rare and scattered (Kausrud et al., 2012). Furthermore, long distance dispersal may be understood as escape from predators and parasitoids. In forest stands with high *I. typographus* density antagonist impact on new infestations (compared to an enemy free space, i.e. caged logs) was found to be doubled in comparison to stands with low *I. typographus* density (Schroeder, 2007). Especially parasitoids which often depend on limited range of host species may reach effective population densities with a considerable time lag. Wermelinger (2002) observed a decreasing survival rate from 46 to 18% in the second year of infestation and ascribed that decrease to increase of predators and parasitoids. Beetles which can escape from those developing antagonist populations may therefore have considerable reproductive advantages. However, the proportional negative influence of predators seems to decrease with increasing aggregation, probably attributed to predator dilution (Aukema and Raffa, 2004).
Generally there exists a fitness trade-off between dispersal and reproduction in which females but also males are punished for flight activity by decreased reproduction (Zera and Denno, 1997). Additionally, to overcome the host defence successfully, bark beetles have to be present in high individual numbers, depending on the tree vigour (e.g. Mulock and Christiansen, 1986). Therefore, dispersing far may rather be an additional evolutionary disadvantage reducing probability of colonisation success and eventually reproductive success. Because *I. typographus* dispersal is non-linear correlated flight in the absence of aggregation pheromones (Byers, 1996; 2000), beetle density in space will decrease with increasing distance from the source (e.g. Anderbrant and Schlyter, 1989). Thus, the probability to encounter conspecifics and therefore increase their own survival probability when attacking vigorous hosts will also decrease. The function of individuals travelling far may rather lie in keeping gene flow high to avoid bottleneck situations. This hypothesis is

supported by the genetic studies of Gugerli et al. (2008) and Stauffer et al. (1999) which indicated a high genetic relatedness among Central European *I. typographus* populations. They concluded that this is most likely caused by high gene flow between populations. Furthermore, beetles capable of covering long distances may also function as pioneers. These beetles, which are the first in finding or boring in suitable host trees (Lehmberg, 2013; Rudinsky et al., 1971; Wermelinger, 2004), should require a longer search time than the beetles which can follow the pheromone source of pioneer beetles.

# General discussion and conclusions

The study presented here concerned several important *I. typographus* life cycle aspects influencing population dynamics. It has long been known that properties like bark anatomy or physiological condition mediate tree susceptibility to bark beetle attack (Baier, 1996; Nihoul and Nef, 1992; Wermelinger, 2004). Here it was shown that nutritional content as well as concentrations of secondary tree metabolites are distributed unequally in Norway spruce. Nutrient content was significantly higher whereas toxic metabolites were concentrated significantly lower in upper stem parts (Tab. 1.1 and Fig. 1.3). This unequal distribution translated in higher offspring fitness of beetles developing in upper tree parts (Fig. 1.6 and Fig. 1.7). Moreover, it was shown that artificial phloem girdling which is also caused by larval feeding leads to an even more unequal nutrient distribution (Tab. 1.2, Tab. 1.3 and Fig. 1.5). These results explain the observed phenomenon that within trees, infestation commences below the crown and then proceeds downwards the stem (Benz and Zuber, 1990; Jahn, 1990; Niemeyer, 1978). They may also explain the large variation in the amount of aggregation pheromone produced between bark beetle individuals (Birgersson et al., 1988; Lehmberg, 2013; Miller et al., 1989; Pureswaran et al., 2000). On the one hand pheromone production includes costs in form of energy reserves (Blomquist et al., 2010; Bohlander and Schopf, 2000; Gries et al., 1990; Hunt and Smirle, 1988) and mortality risk in the early colonisation phase is high when tree defence is still intact. On the other hand, offspring of first colonising individuals have a significant fitness advantage. It was also observed before that lipid content of later emerging beetles decreased (Botterweg, 1982), which in this context would represent beetles developing in lower tree parts. Increasing exploitation competition with infestation intensity adds to effect. It was confirmed in this study (Fig. 2.2) that increasing colonisation densities of *I. typographus* significantly decrease offspring fitness in terms of size, weight and energy reserves, as reported in previous studies (e.g. Atkins, 1975; Botterweg, 1983; Sallé et al., 2005).

*Dispersal capacity*

However, neither short-term starvation nor increased population density had a significant impact on flight capacity of individuals. In the past, several authors argued that especially reduced fitness in high population densities of outbreak situations would reduce flight capacity and therefore dispersal distances and subsequent infestations (e.g. Anderbrant, 1985; Gries, 1985; Williams and Robertson, 2008). The adoption of non-invasive NMR

spectroscopy as novel tool in entomology gave the opportunity to directly analyse flight capacity as function of lipid reserves in this study. It was shown that lipid content was not directly correlated to flight distances achieved on individual level (Fig. 4.4). Beetles rather used their energy reserves with differing efficiency (Fig. 4.5). Moreover, on population level flight distances were skewed to short distances and distributed highly similarly across populations with largely differing developmental conditions (Fig. 5.2). These empirical data can help to improve the knowledge about population dynamics and simulation models thereof, since dispersal is an insufficiently understood trait in the bark beetle life cycle (Preisler et al., 2012). Although not synonymous, flight capacity is one fundamental determinant of dispersal. Predictions that major proportions of *I. typographus* populations travel more than 500 m, 750 m per day or farther than 35 km are largely based on single observations or extrapolations from short distance mark-recapture experiments (see Kausrud et al., 2012 and references therein). The large dataset of empirical data presented here indicates that these assumptions may overestimate *I. typographus* flight potential, at least in the absence of wind drift. Generally bark beetles are assumed to travel in large parts beneath the forest canopy where no wind drift can occur (e.g. Forsse and Solbreck, 1985; Safranyik et al., 1992). In contrast, using radar observation and aerial capture, a high number of *D. ponderosae* was recently found to fly above the canopy up to 800 m high above an outbreak area (Jackson et al., 2008). However, the mechanism and the local vs. general character of this phenomenon remained unclear. The extent to which such phenomena contribute to local population dynamics is not assessable, particularly because beetles drifted in the atmosphere most probably cannot decide where to land but rather depend on thermal constraints. Such a mechanism might rather support new invasions than contribute to local population growth. However, it is important to keep in mind that tethered flight experiments cannot be transferred one-to-one into natural conditions. On the one hand, results of flight mill experiments may underestimate dispersal distances. Beetles might cover much longer distances when drifted by wind (see above). On the other hand, it is more likely that flight mill experiments overestimate the dispersal distances of *I. typographus* shown here. In tethered flight, individuals experience a permanent flight stimulus because they never touch the ground. Furthermore, individuals move in a ‘straight line’ which is highly unlikely in a natural environment where visual cues or semiochemical signals stimulate them to change direction or to land. Also, flight mills support the beetles’ weight so that they have to expend less energy to stay airborne (Byers, 1988; Robertson and Roitberg, 1998). Although this bias was minimized in this study by keeping the beetle mounting (i.e. the silver wire, see Fig. 1.2) flexible. As a result, beetles moved

forward and up when starting flight and thus had to carry the better part of their weight themselves. However, flight mill experiments may still draw a more coherent picture than field experiments. Mark-recapture experiments, which are the alternative used in the field, were usually conducted within short distances only (Jactel and Gaillard, 1991). Recapture success was quite low across all these studies and too low to draw coherent conclusions. For example, Anderbrant (1985) recaptured only 38 of the 20,000 beetles he had marked with fluorescent powder. Franklin and Grégoire (1999) only recaptured less than 0.3% of their marked beetles with trap trees and 6% with pheromone traps. It is highly questionable if conclusions for the whole population can be drawn with such low recapture rates. Moreover, mark-recapture experiments, which mainly recaptured with pheromone traps, rather address the question of aggregation pheromone susceptibility than determining maximal flight distances. In these experiments, it is not clear how much longer individuals would have been able to fly without the artificial pheromone stimulus to aggregate or if susceptible or already infested host trees, which are much more attractive than artificial pheromone, acted as competitors to traps.

*Population dynamics with special regard to climate change*

Overwintering biology is a key factor modulating *I. typographus* population dynamics, as winter mortality can dramatically reduce populations (e.g. Annila, 1969; Faccoli, 2002). It is one of the least understood life cycle stages in *I. typographus* and bark beetles in general (Wermelinger, 2004). Results of this study concerning two major temperature related factors in bark beetle life cycle, namely duration of development (and thereby timing of swarming and voltinism) and winter mortality, may help to evaluate population dynamics more precise, especially with regard to expected warming associated with climate change. Depending on the underlying climate model, an increase of average temperature between 1.1 and 6.4°C is expected by the end of the twenty-first century, relative to the period between 1980 and 1999 (IPCC, 2007). Especially for mid latitudes of Western Europe, phenology models showed that the growing season has expanded approximately by 11 days since the 1970s (Menzel and Fabian, 1999), both by an advanced onset of spring and a forward shift of autumn (Ibáñez et al., 2010; Menzel and Fabian, 1999; Menzel et al., 2006; Parmesan and Yohe, 2003). These changes are mainly attributed to the increase in temperature facilitated by anthropogenic climate change (Menzel and Fabian, 1999; Menzel et al., 2006; Rosenzweig et al., 2008) and expected to increase with predicted global warming (Sparks and Menzel, 2002). As a result, seasonal appearance of insects is expected earlier in a season and period of activity is

prolonged, as shown for numerous butterfly species (e.g. Forister and Shapiro, 2003; Roy and Sparks, 2000) and also for a variety of taxa from plants to vertebrates (Parmesan and Yohe, 2003; Walther et al., 2002). Moreover, univoltine species shifted to bi- or multivoltinism (Altermatt, 2010). These phenomena of earlier onset of development and increased voltinism may facilitate a more rapid population growth and therefore outbreaks of pest species (Neuvonen et al., 1999; Steinbauer et al., 2004; van Asch and Visser, 2007).

Likewise, based on simulation models it has been suggested that *I. typographus* populations might switch to the initiation of an additional generation (Jönsson et al., 2009; Jönsson et al., 2011; Lange et al., 2006). Thus, beside an expected range expansion in latitude and altitude (Walther et al., 2002), which would be restricted to spruce distribution for *I. typographus* (Jönsson et al., 2009), population growth might be more rapid under expected climate change scenarios. However, in the present study it was shown that the developmental stage entering diapause is more important as mortality factor and for post-diapause fitness than temperature. As rightly noted by Jönsson et al. (2009), winter mortality of larvae and pupae can reach up to 100% (Faccoli, 2002; compare also Fig. 3.9 and Fig. 3.10). In the present study it was moreover shown that winter mortality of callow adults with uncompleted maturation feeding can also be up to 50% (Fig. 3.10). Even if callow adults are able to survive winter as indicated before (Baier et al., 2007; Wermelinger and Seifert, 1998), results presented here show that emergence per brood system (Fig. 3.11) and post-diapause fitness of hibernating individuals with uncompleted maturation feeding was significantly reduced (Fig. 3.13). Thus, in a scenario where temperature sums do not suffice for the complete development of an additional generation, population growth may rather be slowed down due to increased winter mortality and reduced post-diapause fitness. Furthermore, proposed models (e.g. Jönsson et al., 2009; Jönsson et al., 2011; Lange et al., 2006) may overestimate *I. typographus* population growth because they assume a shift of the whole population from univoltinism to multivoltinism. Results of the present study are in contrast to such an assumption. Here it was shown that especially under conditions with unpredictable season length large parts of local populations do not initiate an additional generation even when momentary summer conditions are in favour of that (Fig. 3.19).

It has furthermore been suggested that increasing temperatures associated with climate change might be directly correlated to winter survival in bark beetles (Bentz et al., 2010; Preisler et al., 2012). However, diapause in *I. typographus* can be seen rather as reproductive than as total developmental diapause. Lower temperature threshold for maturation feeding of callow adults is quite low with 3.2 °C (Wermelinger and Seifert, 1998) and during hibernation also

phases of activity and feeding occur (Koštál et al., 2011). Additionally it is known that both energy reserves (Botterweg, 1982; Kraußе-Opatz et al., 1995; compare also Tab. 3.1 and Fig. 3.12d, Fig. 3.18d) and also fecundity (e.g. Ellers and van Alphen, 2002) of insects can decrease significantly with diapause duration. Metabolic rate is indeed depressed in diapausing insects. But it still remains responsive such that it increases with temperature (Hahn and Denlinger, 2007). As a result, consumption of energy reserves will be higher when average winter temperatures increase. Thus, higher temperature may mediate lower diapause survival rates through increased costs of metabolism maintenance (e.g. Irwin and Lee Jr., 2000; Thompson and Davis, 1981). In freezetolerant species this effect of energy saving may even be amplified. For larvae of the goldenrod gall fly *Eurosta solidaginis* (Diptera, Tephritidae) it has been shown that increased likelihood of freezing correlates with higher survival and greater post-diapause fecundity (Irwin and Lee Jr., 2000; 2003; Williams et al., 2003). As yet, there is a lack of studies analysing overwintering success in terms of survival, post-diapause energy reserves and fecundity as function of winter temperature regimes in bark beetles. Thus, higher winter temperatures have a yet unknown effect on *I. typographus*. On the one hand, less severe conditions during winter may decrease mortality caused by freezing as suggested. On the other hand there are indications from other insect taxa that higher temperatures during diapause may increase mortality and decrease post-diapause fitness due to increased metabolic rates. Bark beetles may face an additional risk when summer and autumn are prolonged through increased temperatures as reported recently (Luterbacher et al., 2007). They rely on cryoprotectants to prevent freezing of body water during winter diapause (Koštál et al., 2011; Koštál et al., 2007). These compounds have to be built well before winter (Hahn and Denlinger, 2007; Koštál, 2006). In *I. typographus* day length and temperature are the cues triggering hibernation onset (Doležal and Sehnal, 2007a; Schopf, 1985; 1989). Recently it has been shown that high temperatures can override the signal of decreasing day length (Doležal and Sehnal, 2007a). Thus, a missing signal due to increased temperatures may prevent a timely accumulation of cryoprotectants and therefore increase mortality.

In conclusion, without further empirical studies development of bark beetle population dynamics with regard to predicted increases in temperature remains highly speculative. It is more likely that expected changes in variability and increase of extreme weather event occurrence (Diffenbaugh et al., 2005; Luterbacher et al., 2007; Meehl and Tebaldi, 2004; Netherer and Schopf, 2010) add significantly to bark beetle population growth. Extreme drought periods and shifts of precipitation patterns, as experienced in 2003 (Rebetez et al., 2006; Rouault et al., 2006), as well as severe storms such as in the 1990s (Wermelinger,

2002) and 2000s (Klaus et al., 2011) provide a vast number of weakened or broken host trees. These are key factors favouring bark beetle population growth (Wermelinger, 2004) and thus may facilitate an increasing number of mass outbreaks under such conditions.

*Bark beetle management*

Especially *I. typographus* overwintering behaviour highlights difficulties of forest management measures. Currently, the removal of suitable brood material like dead or wind thrown trees and sanitation logging of infested trees is considered the most efficient management method (Wermelinger, 2002; 2004). Since crown discolouration of infested spruces from green to brown can occur with a considerable time lag, locating beetle frass at tree trunks during dispersal phases is currently the most reliable method of identifying infestations in a timely manner. These methods require high effort and man power and produce enormous costs (Heurich et al., 2001; Koštál et al., 2011; Wermelinger, 2002). Still, large outbreaks could not be prevented in the last decades (Wermelinger, 2002). Therefore proper assessment of infestation risks is crucial for decision making in forest management.

Sanitation measures might also interfere with bark beetle antagonist populations (Kausrud et al., 2012; Wermelinger, 2002) and Kautz et al. (2013) recently showed that especially sun exposed forest edges created by sanitation logging are susceptible to new bark beetle infestation. The present study identified additional problems of sanitation logging. A considerable number of beetles may be hibernating within fallen bark on the ground (Fig. 3.2 and Fig. 3.16). Thus, when trees infested during the summer swarming period are not recognised immediately and are not removed before winter, a great number of beetles can remain in the forest with fallen bark. Moreover, the results highlight the importance of removing infested trees even when the infestation occurred in a previous infestation period. That is because a significant proportion of the population might initiate hibernation already in summer and may not emerge until the following spring (Fig. 3.7 and Fig. 3.19). However, the problem remains that beetles stay in the forest within fallen bark if trees are not carefully removed or the fallen bark is not burned or shredded.

Moreover, for the establishment and management of protected areas without control measures proper assessment of possible socioeconomic risks for surrounding economically used forests is essential (Netherer and Nopp-Mayr, 2005). On the one hand bark beetles are considered as important rejuvenation agent (McFarlane and Watson, 2008) and keystone species in natural forest ecosystems, facilitating biodiversity by providing dead wood and sunlit gaps (Müller et al., 2008). On the other hand, bark beetle outbreaks in protected areas can change the forest

structure drastically within a short time frame and may flash over to commercial forests (McFarlane and Watson, 2008; Müller et al., 2008; Müller and Job, 2009). Thus, the application of control measures in protected areas is controversially debated by the public, politicians and scientists (Koštál et al., 2011; Müller et al., 2008). The result presented here that more than 50% of studied *I. typographus* populations were not capable of flying farther than 500 m (Fig. 5.2) strengthens the results of Kautz et al. (2011) who showed that 95% of new infestation spots in the Bavarian Forest National Park between 1990 and 2010 occurred within 500 m of previous infestations. Though not directly comparable, due to the fact that Kautz et al. (2011) did not consider where beetles causing new infestations originated from, it is shown here that a large majority of *I. typographus* populations will only be able to disperse over short distances. Taking into account that it is quite unlikely that beetles in nature disperse in a straight line as in the laboratory experiments presented here, both results match very well. Thus, measures such as 500 to 1500 m management zones encircling protected, unmanaged areas like practised for example in the Bavarian Forest National Park (Bavarian Forest National Park Administration, 2010; Bavarian State Government, 2007; Heurich et al., 2001) are very likely to drastically minimise infestation risks in surrounding economically used forests, given that enough host trees are present in this management zone.

*Conclusions*

Summing up, this study illustrates large variation in various life cycle traits and behaviour in *I. typographus* populations, such as hibernation site, timing of hibernation onset, flight capacity and efficiency of energy usage, respectively. These findings add to variation in other traits between individuals of this species, such as pheromone production (Birgersson et al., 1988; Lehmberg, 2013; Miller et al., 1989; Pureswaran et al., 2000). This multifaceted plasticity facilitates spreading of mortality risks in *I. typographus* populations. Therefore, it enables the species to persist in, and rapidly adapt to, unpredictable and changing environments. Such phenotypic plasticity is a function of environmental lability, and its persistance strongly depends on migration between populations (reviewed in Chown and Terblanche, 2007), which illustrates an additional function of individuals dispersing far. Furthermore, the multifaceted plasticity highlights the importance of sound empirical data for modelling population dynamics and risk assessment in forest management.

# Summary

Bark beetles (Coleoptera, Curculionidae, Scolytinae) are of fundamental importance for forest ecosystems due to their ecosystem function of facilitating fungal, insect and plant diversity by creating dead wood and forest gaps. However, aggressive bark beetle species, which are able of attacking vigorous trees, can kill extensive areas of trees in mass outbreak situations in economically used forests. Thus, knowledge about factors influencing population dynamics is fundamental for both ecologists and foresters. In Europe, the spruce bark beetle *I. typographus* L. is the most aggressive species. Its larvae and adults feed almost exclusively on secondary phloem of Norway spruce *Picea abies* (L.) Karst. (Coniferales, Pinaceae). In this dissertation, factors influencing individual fitness were studied, with emphasis on conditions of larval development. Body weight, size, energy (i.e. lipid) reserves and flight capacity are used as fitness parameters throughout this study. The dissertation is divided into five chapters. The focus of the first three chapters is on the influence of host tree metabolites, population density and overwintering biology on individual fitness. The fourth chapter deals with the relationship of lipid reserves and flight capacity on an individual level. Distribution of flight capacity on population level is analysed in the fifth chapter. Implications for population dynamics, potential effects of expected climate change and consequences for forest management are discussed in a synopsis of the different chapters.

In the first chapter it is shown that nutrients are concentrated significantly higher and toxic metabolites significantly lower in upper stem parts within Norway spruces. Artificial phloem girdling, which was used to simulate larval feeding activity, even amplified the effect of inhomogeneous metabolite distribution. The unequal distribution seemed to translate into beetle fitness, as individuals emerging from lower stem parts were significantly more lightweight than those emerging from upper parts. This fitness advantage of individuals developing in upper stem parts explains the observed phenomenon that infestations within trees commence below the crown and then proceed downwards the stem.

In the second chapter the influence of population density on individual fitness was tested. Beetles developing in high population density were significantly more lightweight, smaller and had significantly lower lipid reserves than beetles from low densities. However, flight capacity was not affected by population density. Lipid reserves were in large parts depleted

after flight, showing that they play a major role in fuelling *I. typographus* flight activity. Nevertheless, flight distances did neither correlate with beetle size nor weight.

The third chapter highlights *I. typographus* overwintering biology. Winter survivability and individual fitness of surviving beetles was considerably higher when individuals entered winter as adults with completed maturation feeding. Larvae, pupae and probably even callow adults experienced high mortality rates. As a result, population structure is shifted towards adults over winter, which may contribute to synchronize spring swarming and host attack. On the other hand this can severely decelerate population growth, especially in cases when an additional generation is established late in a season and cold sensitive developmental stages overwinter.

The primary overwintering location of *I. typographus* was the host tree. Soil hibernating individuals mainly reached the ground within falling bark. Energy reserves were more depleted in beetles which overwintered in trees. Nevertheless, apart from influencing energy reserves differently, both overwintering sites have other advantages and mortality risks such as differences in fungal or predator diversity and abundance. The mixed hibernation sites, mediated by a passive mechanism, spread winter mortality risks within populations and thus may contribute to local population stability.

Furthermore, variable fractions of the studied population did not establish an additional generation in a season, even when environmental conditions were in favour of that. This life-history strategy of partly establishing an additional generation to enhance population growth and partly initiating hibernation early in a season to enhance winter survivability can account substantially for population stability in an unpredictable environment. It adds to the phenomenon of mixed hibernation sites described above in spreading the risk of winter mortality and thus in sustaining local populations.

In the fourth chapter, the relationship between lipid reserves and flight capacity is analysed on an individual level. To bypass killing of individuals required when using conventional lipid analysis methods, a non-invasive method for insect lipid content analysis (i.e. nuclear magnetic resonance spectroscopy) was applied in cooperation with the Institute of Medical Engineering, TUM. This method provided the opportunity to measure lipid contents before and after flight experiments in the same insect individuals. Thus, flight capacity could be analysed directly as function of lipid reserves and consumption. It was demonstrated that

flight capacity is in large parts independent of initial lipid reserves. Individuals capable of flying long distances rather were more efficient in the usage of their reserves.

In the fifth chapter it is demonstrated that on population level, distributions of flight capacity do not differ between populations. Independent of lipid reserve distribution within populations, flight distances were skewed to short distances and were best fitted by a negative power law function in all populations studied, with about half of a population flying only 500m or less.

In summary, in this dissertation it is demonstrated that conditions of larval development severely influence fitness in terms of energy reserves. Individual fitness is decreased by decreased nutritional content and increased toxic metabolites of host trees, increased population density and when immature developmental stages enter hibernation. Decreased fitness (i.e. lipid reserves) may influence population dynamics through its negative impact on host selection behaviour, pheromone production, the ability to detoxicate secondary host tree metabolites or fecundity. However, flight capacity is largely independent of conditions of larval development. In species like *I. typographus*, which require high densities of attacking individuals to overwhelm defence of vigorous host trees, dispersing far would decrease spatial density of individuals and therefore decrease individual survivability and colonisation success.

Moreover, this study illustrates large variations in various life cycle traits and behaviour in *I. typographus* populations, such as hibernation site, timing of hibernation onset, flight capacity and efficiency of energy usage, respectively. This multifaceted plasticity facilitates spreading of mortality risks and therefore supports *I. typographus* to persist in and rapidly adapt to unpredictable and changing environments.

# Zusammenfassung

Borkenkäfer (Coleoptera, Curculionidae, Scolytinae) spielen eine wichtige Rolle in Waldökosystemen. Dadurch, dass sie Totholz und Lichtungen schaffen, leisten sie einen wesentlichen Beitrag zur Erhaltung von Pilz-, Insekten- und Pflanzendiversität. Einige Arten, die fähig sind gesunde Bäume zu besiedeln, können in wirtschaftlich genutzten Wäldern jedoch erheblichen Schaden verursachen. Aus diesen Gründen ist das Wissen über Faktoren, die ihre Populationsdynamik beeinflussen, sowohl für Ökologen als auch für Förster unentbehrlich. Die aggressivste Art in Europa ist der Buchdrucker *Ips typographus*. Sowohl Larven als auch Adulte ernähren sich fast ausschließlich von Phloem der Gemeinen Fichte *Picea abies* (L.) Karst. (Coniferales, Pinaceae). In dieser Dissertation wurden Faktoren untersucht, die die individuelle Fitness von Buchdruckern beeinflussen. Der Fokus lag dabei auf unterschiedlichen Bedingungen der Larvalentwicklung. Gewicht, Größe, Energiereserven und Flugleistung wurden dabei als Fitnessparameter benutzt. Die ersten drei Kapitel beschäftigen sich mit dem Einfluss von Wirtsbaum-Metaboliten, Populationsdichte und Überwinterung. Im vierten Kapitel wurde der Zusammenhang zwischen Fettreserven und Flugkapazität auf Individuen-Basis untersucht, während im fünften Kapitel die Verteilung der Flugkapazität zwischen verschiedenen Populationen verglichen wurde. Am Ende werden die Ergebnisse in einer Zusammenschau im Hinblick auf ihre Bedeutung für die Populationsdynamik, die erwartete Klimaerwärmung und das Forst-Management diskutiert.

Im ersten Kapitel wird gezeigt, dass Nährstoffe in oberen Stammabschnitten von Fichten signifikant höher konzentriert sind als in unteren, während toxische Sekundärmetabolite in höheren Abschnitten signifikant niedriger konzentriert sind. Künstliche Ringelung der Bäume, die die Fraßaktivität der Larven simulierte, verstärkte die Ungleichverteilung der Bauminhaltsstoffe zusätzlich. Die inhomogene Verteilung drückte sich auch in der individuellen Fitness der Käfer aus. Individuen, die sich in unteren Stammabschnitten entwickelten, waren signifikant leichter als solche aus oberen. Dieser Fitness-Vorteil von Individuen die sich in oberen Stammabschnitten entwickeln erklärt den Befallsverlauf, der innerhalb eines Baumes in der Regel am Kronenansatz beginnt und sich dann nach unten fortsetzt.

Im zweiten Kapitel wird der Einfluss der Populationsdichte auf die individuelle Fitness untersucht. Käfer, die sich in hoher Populationsdichte entwickelten waren signifikant leichter

und kleiner und hatten signifikant weniger Fettreserven. Die Flugkapazität dieser Käfer war jedoch nicht verringert. Die erreichten Flugweiten korrelierten weder mit Käfergewichten noch mit ihren Größen, allerdings waren die Lipidreserven nach dem Flug zu einem großen Teil verbraucht.

Das dritte Kapitel beschäftigt sich mit verschiedenen Aspekten der Überwinterungsbiologie des Buchdruckers. Die Überlebenswahrscheinlichkeit von Individuen und ihre individuelle Fitness waren signifikant höher, wenn sie ihren Reifungsfraß vor dem Winter abgeschlossen hatten. Die Mortalitätsrate von Larven, Puppen und auch jungen adulten Käfern war sehr hoch. Dadurch wird die Populationsstruktur über Winter hin zu adulten Individuen verschoben. Dieser Effekt trägt zu einer Synchronisation der Frühjahrsdispersion und des Wirtsangriffes bei. Auf der anderen Seite kann das Populationswachstum dadurch stark verringert werden, besonders dann, wenn eine zusätzliche Generation zu spät in einer Saison begonnen wird und dadurch besonders kältesensitive Entwicklungsstadien überwintern müssen.
Weiterhin wurde gezeigt, dass der primäre Überwinterungsort des Buchdruckers der Wirtsbaum ist. Individuen die im Boden überwinterten, gelangten hauptsächlich in herabfallenden Rindenstücken dorthin. Die Energiereserven der stammüberwinternden Käfer waren allerdings zu einem größeren Teil verbraucht als die der bodenüberwinternden. Der Stamm als Überwinterungsort hat jedoch andere Vorteile, wie zum Beispiel eine geringere Diversität und Abundanz von Räubern oder Pilzen. Der gemischte Überwinterungsort, verursacht durch den passiven Mechanismus der herabfallenden Rinde, trägt deshalb dazu bei, das Mortalitätsrisiko innerhalb von Populationen zu streuen.
Unterschiedlich große Anteile der untersuchten Populationen begannen keine zweite Generation innerhalb einer Saison, selbst wenn dies aufgrund der äußeren Bedingungen erwartet worden wäre. Diese Strategie, teilweise eine zusätzliche Generation zu beginnen um die Fortpflanzungsrate zu erhöhen, und teilweise die Überwinterung schon nach der ersten Generation einzuleiten um das Mortalitätsrisiko zu verringern, trägt zusätzlich zur Stabilität von Populationen unter nicht vorhersagbaren Umweltbedingen bei.

Im vierten Kapitel wird der Zusammenhang zwischen Lipidreserven und Flugkapazität auf Individuen-Basis analysiert. In Zusammenarbeit mit dem Zentralinstitut für Medizintechnik der TUM wurde eine kernspinresonanztomographische Methode angewandt, mit Hilfe derer der Lipidgehalt von Insekten nicht-invasiv bestimmt werden kann, wodurch die getesteten

Individuen zur Lipidanalyse nicht getötet werden mussten. Lipidreserven konnten dadurch erstmals vor und nach dem Flug analysiert werden, und damit auch der Lipidverbrauch pro Flugstrecke. Mit dieser Methode konnte gezeigt werden, dass die Flugleistung unabhängig vom ursprünglichen Lipidgehalt ist. Käfer, die in der Lage waren weiter zu fliegen, nutzten ihre Reserven effizienter.

Im fünften Kapitel wird gezeigt, dass sich die Verteilungen der erreichten Flugweiten auf Populationsebene nicht unterscheiden. Unabhängig von Verteilungen der Energiereserven innerhalb der untersuchten Populationen waren die Flugkapazitäten immer zu kurzen Weiten hin verschoben, wobei circa die Hälfte einer Population Weiten von 500 m oder weniger erreichte.

Zusammengefasst zeigen die Ergebnisse dieser Dissertation, dass die Bedingungen während der Larvalentwicklung die individuelle Fitness signifikant beeinflussen. Diese wird durch geringere Nährstoff- und höhere Giftstoffkonzentration des Wirtsbaumes, durch erhöhte Populationsdichte und wenn junge Entwicklungsstadien überwintern deutlich verringert. Verringerte Fitness beeinflusst die Populationsdynamik durch ihren negativen Effekt auf Wirtsbaumwahl, Pheromonproduktion, die Fähigkeit zur Entgiftung von sekundären Wirtsbaum-Inhaltsstoffen und Reproduktionsrate. Die Dispersionsfähigkeit ausgedrückt als Flugkapazität wird von unterschiedlichen Entwicklungsbedingungen jedoch kaum beeinflusst. Bei Arten wie *I. typographus*, die auf eine hohe Individuendichte zur Überwindung der Wirtsbaumabwehr angewiesen sind, würde eine weite Dispersion zu einem Verdünnungseffekt der Individuen führen und dadurch die individuelle Überlebenswahrscheinlichkeit und den Besiedelungserfolg verringern.
Die vielfältige Plastizität, die in dieser Dissertation für verschiedene Lebenszyklus- und Verhaltens-Bereiche von *I. typographus* gezeigt wurde, trägt entscheidend dazu bei, dass Mortalitätsrisiken innerhalb von Populationen gestreut werden und sich die Art somit schnell an sich verändernde Umweltbedingungen anpassen kann.

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# Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt (promotionsführende Einrichtung) der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:

Ecophysiology of the European spruce bark beetle (*Ips typographus* L.): Factors affecting individual fitness, dispersal and population dynamics

in Lehrstuhl für Tierökologie

(Fakultät, Institut, Lehrstuhl, Klinik, Krankenhaus, Abteilung)

unter der Anleitung und Betreuung durch: Prof. Dr. Reinhard Schopf

ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Abs. 6 und 7 Satz 2 angegebenen Hilfsmittel benutzt habe.

☑ Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

☑ Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

☐ Die vollständige Dissertation wurde in .....

veröffentlicht. Die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt (promotionsführende Einrichtung) hat der Veröffentlichung zugestimmt.

☑ Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

☐ Ich habe bereits am .....

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die Zulassung zur Promotion beantragt mit dem Ergebnis:

Die öffentlich zugängliche Promotionsordnung der TUM ist mir bekannt, insbesondere habe ich die Bedeutung von § 28 (Nichtigkeit der Promotion) und § 29 (Entzug des Doktorgrades) zur Kenntnis genommen. Ich bin mir der Konsequenzen einer falschen Eidesstattlichen Erklärung bewusst.

Mit der Aufnahme meiner personenbezogenen Daten in die Alumni-Datei bei der TUM bin ich

☑ einverstanden ☐ nicht einverstanden.

Heidelberg, 25.04.2013

(Ort, Datum, Unterschrift)