Mosquito control: Improving aquatic risk assessment and efficiency of mosquito control practices

Dissertation

Zur Erlangung des akademische Grades

Doctor of Philosophy (PhD)

Vorgelegt der

Naturwissenschaftlischen Fakultät I

Biowissenschaften

Der Martin-Luther-Universität Halle-Wittenberg

Von

Frau Alvine Larissa MEYABEME ELONO

Geb. am: 29.06.1975 in Jaunde

Gutachter:

1. Prof. Dr. Hans-Jörg Ferenz
(Institute of Biology, Martin-Luther-Universität Halle-Wittenberg, Germany)
2. Prof. Leon Blaustein
(Institute of Evolution and Department of Evolutionary & Environmental Biology, University of Haifa, Israel)
3. PD Dr. Matthias Liess
(Helmholtz Centre for Environmental Research-UFZ, Leipzig, Germany)

Öffentliche Verteidigung am: 23.05.2011

List of papers

This thesis is based on the following papers, which are referred to in the text as Chapters:

II Meyabeme Elono, A.L., M. Liess, and S. Duquesne. 2010. Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetland areas in Germany. J Vector Ecol (*in press*).

III Meyabeme Elono, A.L., S. Duquesne, K. Foit and M. Liess. Population response of *Culex pipiens* to thiacloprid is altered by interspecific interactions. Ecotoxicology (*in preparation for submission*).

IV Meyabeme Elono, A.L., M. Liess, and S. Duquesne. Invertebrate density sustaining the efficiency of Bti based VectoBac in mosquito control, a case study in Cameroon. Am J Trop Med Hyg (*in preparation for submission*).

Conferences

Meyabeme Elono, A.L., S. Duquesne, and M. Liess. 2009. Aquatic invertebrate controlling dynamics of mosquito larvae in natural ecosystems. Oral presentation, 5th International Congress of the Society of Vector Ecology (SOVE), Antalya-Belek, Turkey.

Meyabeme Elono, A.L., M. Liess, and S. Duquesne. 2010. Invertebrate density sustaining the efficiency of Bti based VectoBac in mosquito control, a case study in Cameroon. Poster, 17th Conference of the European Society of Vector Ecology (ESOVE), Wroclaw, Poland.

Mosquitoes. “No animal on earth has touched so directly and profoundly the lives of so many beings’” (Spielman & D’Antonio 2001)

ACKNOWLEDGEMENTS

It is a pleasure to thank the many people who made this thesis possible. A special mention should be made to the German Academic exchange Service (DAAD) and the Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany, that supported financially this research work.

I am deeply grateful to Prof. Dr. H.-J. Ferenz, my mentor, for his support of the PhD thesis at the University "Martin-Luther-Universität Halle-Wittenberg" and for his valuable assistance.

I owe my sincere gratitude to Dr. PD Matthias Liess and Dr Sabine Duquesne who gave me the opportunity to work with them in the Helmholtz Centre for Environmental Research-UFZ Leipzig. They helped and assisted me through all steps of this work.

I wish to thank Kaarina Foit, Dr. Mira Kattwinkel, and Dr Mikhail Beketov for their guidance in statistical analysis; and in addition Ida, Iris, Jeremias, Nadine, Nathalie, Oliver, Saskia, Sinje, and all other colleagues for interesting discussions and good times.

I am grateful to Mrs Ingrid Ränker and Mr Klaus Seyfarth for their untiring assistance during laboratory tests.

I warmly thank Cléo Mahouva, Wadinga Fombat, Kaarina Foit, and Mira Kattwinkel for helping me getting through difficult times and care they provided.

My special gratitude is due to my siblings Alain Ebogo, Rodrigue Menguele, Martial Menguele, Florence Ada, and Sandra Elono for their affective support.

I owe my loving thanks to my lovely husband, Nestor Meyong, and my lovely children, Franck, Stéphanie, and Michel. They have suffered from my absence during my research abroad. Without their encouragement and understanding, it would not have been possible for me to realise this work.

Lastly, and most importantly, I wish to thank my parents, Marguérite M. Menguele and Luc B. Menguele. They gave birth to me, raised me, supported me, taught me, loved me, and took care of my family during my stay abroad. To them I dedicate this thesis.

CONTENTS

ABBREVIATIONS

Bti	*Bacillus thuringiensis* var *israelensis*
DDT	Dichloro-diphenyl-trichloroethane
DO	Dissolved oxygen
EC	Electric conductivity
EPA	Environmental protection agency
IRS	Indoor residual spraying
ITN	Insecticide treated bednets
LC50	Median lethal concentration
MA	Massachussetts
NTU	Nephelometric turbidity units
OECD	Organisation for Economic Co-operation and Development
PCA	Principal component analysis
PRC	Principal response curves
RDA	Redundancy analysis
RFU	Relative fluorescence units
SCOR	Scientific committee on oceanic research
TDS	Total dissolved solids
UNESCO	United nations educational, scientific and cultural organization
USA	United States of America
USEPA	United States Evironmental Protection Agency
WHO	World Health Organization

SUMMARY

Mosquitoes can be nuisance, pests, and vectors of dangerous diseases such as malaria and West Nile Virus. The present thesis improves the ecological knowledge for mosquito control and aquatic risk assessment of larvicides. After the general introduction (Chapter I), the relationships between mosquito larvae and associated invertebrates including predators and competitors (natural enemies) are investigated (Chapter II). The study took place in temporary aquatic ecosystems of central Germany, in areas with high prevalence of mosquitoes. We found that larval abundance of *Aedes* spp., which was the dominant species of mosquitoes, was negatively correlated with the abundance of competitors for food, and to lesser extend with the abundances of intraguild predators and strict predators. The study in Chapter III investigates the ecological risk and the efficiency of thiacloprid, as a relatively new neonicotinoid insecticide for larval control of mosquitoes in an outdoor microcosm experiment. The study included one setup with an invertebrate community added and another setup without the invertebrate community. The two setups were left to be colonised by local populations of *Culex pipiens* before they were treated three times with thiacloprid concentrations of 0.1 µg/L, 1 µg/L, and 10 µg/L. We found that exposure to thiacloprid showed no effect at the two lower concentrations. Furthermore, the larval abundances of *Culex pipiens* were inversely affected in the setups without and with community added. In the setup without a community added, the abundance of larval *Cx. pipiens* decreased temporarily in the concentration of thiacloprid of 10 µg/L; whereas in the setup with community added, a temporary increase of larval *Cx. pipiens* was coupled to the reduction of potential natural enemies (e.g. Ostracoda and

Copepoda). This chapter underlines the importance of direct and indirect effects for risk assessment of pesticides, and stresses the importance of multispecies tests. In Chapter IV, the regulation potential of natural enemies for mosquito control is tested in a field experiment. The study took place in man-made temporary ponds of a natural wetland in a country at high risk of mosquitoes in terms of public health problems, namely Cameroon. The biological pesticide VectoBac (based on *Bacillus thuringiensis* var *israelensis*) was used as an insecticide. Treatments including invertebrate community alone, VectoBac alone, and the combination of both Vectobac and different density levels of invertebrate community, were compared. The findings demonstrated that the efficiency of VectoBac was highly increased in association with high densities of natural enemies. In conclusion, this thesis provides suggestions about how to use natural enemies to reinforce mosquito control strategies and mitigate the ecological burden associated.

Chapter I

General introduction

1. Mosquitoes and mosquito-borne diseases

Mosquitoes are insects of the order Diptera, family Culicidae. These insects serve for the transmission of parasitic and viral infections to millions of people in the world. Mosquito-borne infections are among the biggest killers at the time and the threat is the most severe in African countries (Sachs & Malaney 2002, Reiter 2010). For example malaria, which is transmitted to humans by *Anopheles* species, reaches 350 to 500 million clinical cases every year resulting in over one million deaths; Africa alone counts for more than 80% of the subsequent mortality registered (WHO, 2005). Dengue and yellow fever are transmitted to humans by *Aedes* species. The number of infections reported for these two diseases are currently estimated at 50 million and 200 000 cases per year, respectively (Reiter 2010); and 30 000 deaths are accounted yearly for yellow fever. Filariasis, encephalitis, and other viral diseases pass also via mosquito bites. The geographical risk of transmission of these diseases is increasing. For example, typical tropical mosquitoes can occur and infect people in temperate regions; the case of *Aedes albopictus* in Ravenna (Italy), which infected more than 200 people with Chikungunya fever during the summer 2007 (Weissman 2008, Thiboutot et al. 2010). This episode of Chikungunya fever was the first outbreak of this arbovirusis in Europe (Weissman 2008). Apart from public health problems, other

main damages caused by mosquitoes in countries at high risk are economical losses including the reduction in tourism.

The life cycle of mosquitoes requires two types of environments; the immature stages (i.e. eggs, larvae, and pupae) develop in aquatic habitats whereas adults live in terrestrial ecosystems. Mosquitoes can lay 50 to 500 eggs onto the water surface (e.g. *Anopheles* and *Culex* species) or on moist soil liable to flooding (*Aedes* and *Ochlerotatus* species) (Becker et al. 2003). Except for larvae of *Toxorhynchites* which prey on other insects, larvae of mosquitoes mostly feed on bacteria, algae, and detritus. All adults utilise sugar from plant nectar as source of food to survive. Mosquito pupae do not feed and are mobile unlike those of many other insects. Female mosquitoes are ready to mate immediately following emergence to adults; in the contrast, males require some days so that the morphological maturation of their accessory reproductive gland is completed (Takken et al. 2006). Many mosquitoes need an obligatory blood meal to produce eggs; adult females of most species bite and suck blood from humans and animals to get iron (Zhou et al 2007) and other nutrient components (McMeniman et al. 2011) for the completion of their ovarian development. It is through this blood meal process that mosquitoes can transmit diseases from an infected subject to an uninfected subject causing public health problems. Hence, the control of mosquitoes is an essential concern.

2. Mosquito control: State of art

Since the first discovery of a disease borne pathogen in mosquitoes (Ross 1897), numerous tools, including various insecticides, have been exploited for mosquito control worldwide (Barnard 2003, Ramirez et al. 2009, Reiter 2010).

Because insecticides currently available in the market are not sufficient to face the delivery from the mosquito burden (Hemingway et al 2006), the great need of new insecticides with new mode of action was recently raised as an urgent tool (Hemingway et al 2006, Mendis et al. 2009).

Control strategies may be directed to controlling adults of mosquitoes or their immature stages at the breeding sites. Adult control of mosquitoes is commonly practiced and relies mainly on insecticide-treated bed nets (ITN) in countries of Africa, South-East Asia, and Western Pacific, and on indoors residual spraying (IRS) in European regions (WHO 2008). ITN and IRS do not affect exophilic and exophagic mosquitoes or those that have reduced their tendency to remain and rest in treated houses. Thus, owing to the fact that adults of mosquitoes can fly and escape intervention measures, control strategies targeting their larval stages (which are restricted to their known aquatic ecosystems) can be more effective and considered as a priority (Killeen et al 2002).

To date, various chemical and biological agents are used for controlling larval stages of mosquitoes. These agents have different mode of action and the magnitude of impacts on communities which are associated to mosquito larvae differ as well. For example, chemical insecticides (e.g. the organophosphate temephos) are more toxic to non-target organisms than insect growth regulators (e.g. methoprene) (Mortimer & Chapman 1995). Larvicides based on the bacterium *Bacillus thuringiensis* var *israelensis* (Bti) are among the safest pesticides for larval control of mosquitoes of the moment (Boisvert 2005). However, Bti becomes quickly unavailable to the filter feeding mosquito larvae due to the fast adsorption of spores to the sediments. Moreover, it was shown that Bti can also exhibit a toxic activity to non-target taxa

such as Chironomidae, *Chlorella sp.*, and *Closterium sp.* (Boisvert & Boisvert, 2000). Thus, research to find safer approaches for the management of mosquito control is crucial.

3. Risk assessment of mosquito control agents

The release of insecticides in aquatic environments can cause direct effects on organisms (e.g. mortality) and indirect effects on organisms through altered community structure (Poulin et al. 2010). The environmental risk of insecticides is usually estimated based on direct effects derived from laboratory tests on single species. In those tests, only the reduction of organism abundance as a consequence of an increase in mortality or a reduction in fecundity is considered (OECD 2000, WHO 2010). However indirect effects inducing changes in behaviour, competition, and predation/grazing rate are known to affect substantially natural communities and food webs (Fleeger et al. 2003). One typical example about mosquito control agents is the significant mortality of cats and a subsequent increase in rat populations following the spraying of DDT and dieldrin in villages in Borneo (Clements & Newmann 2002). Another example is the increased mortality in swallow's nestlings, due to the elimination of their favourite food sources (i.e. Nematocera) following the larval control of mosquitoes using Bti-based VectoBac in the Rhone Delta, France (Poulin et al 2010). So, insecticides may alter biological interactions by reducing for instance predation rates/influence on a given prey or prey availability for a given predator. Such indirect effects may eventually lead towards communities dominated by species that are not affected by the pesticides or those having a better recovery potential such as mosquitoes.

Mosquitoes have a short generation time and the recolonisation of aquatic ecosystems by these insects is facilitated by their terrestrial adults. Morover, it was shown that female mosquitoes oviposit preferentially in habitats with fewer competitors (Mokany & Shine 2003) and fewer predators (Stav et al. 2000, Eitam et al. 2002, Blaustein et al. 2004). Moreover, predators and competitors (hereafter natural enemies) can reduce the survival of mosquitoes by preying on larvae or competing for the same food sources (Knight et al. 2003, Marten & Reid 2007, Duquesne et al. 2010). A decrease in aquatic biodiversity as a result of insecticide treatment can be followed by resurgence or secondary outbreaks of mosquitoes due to losses of potential natural enemies. In addition to individual tests, studies on risk assessment at the community level are essential to provide guidance for the most appropriate mosquito control management.

4. Objectives and aims of the thesis

The present research work proposes to (i) determine ecological communities associated with mosquito larvae in typical breeding sites by characterising especially natural enemies, and (ii) assess the modifications generated in the structure of these communities after application of larvicides and how this ultimately affects mosquitoes.

The knowledge gathered here will contribute to the development of an ecological approach for an improved risk assessment of mosquito larvicides, and the identification of the main ecological elements that can support successful and sustainable mosquito control practices.

4.1. Improving aquatic risk assessment

The ecological effects of thiacloprid on targets (mosquito larvae) and non-target (associated invertebrate taxa) communities were investigated. Thiacloprid belongs to the relatively new class of neonicotinoid insecticides. The study was carried out in artificial water bodies of the Central Germany (Central Europe); simulating ecosystems where mosquitoes are of concern (i.e. Rosslau, Spreewald, and Leipzig, 2007). Distinct field situations are represented. For example we constitute enemy-free and enemy-enriched water bodies which correspond to the conditions in newly formed and aged pools, respectively.

The aim is to define appropriate threshold levels of thiacloprid for larval control of mosquitoes and provide basic knowledge to support and improve the risk assessment of the potential new insecticides.

4.2. Improving ecological knowledge to support mosquito control methods

As a part of the objective 4.1., changes in the abundances of mosquito larvae and their associated invertebrate taxa were recorded following larvicide treatments in an outdoor microcosm experiment (Germany, 2007) and a field study (Cameroon, 2008). A particular attention was given to the abundances of predators and competitors (natural enemies), and their potential influence for regulating larval populations of mosquitoes in the post-treatment period. For instance, the abundances of natural enemies and mosquito larvae can decrease simultaneously after treatment. However, if the development of natural enemies is exclusively dependent on aquatic environments, the re-growth of their populations is delayed; and such processes can lead to undesirable recovery of mosquitoes whose adults are terrestrial.

The aim is to improve the knowledge to provide recommendations for the most efficient mosquito control practices and to interpret data collected from the field.

4.3. Improving mosquito control practices

The capacity of natural enemies of mosquito larvae for supporting the larval control by the biopesticide *Bacillus thuringiensis* var *israelensis* (Bti) is studied. This investigation is conducted in Cameroon (Central Africa), an area of high concern of mosquito-borne diseases causing public health problems. Treatments using natural enemies at different density levels are exploited in a semi field investigation.

The aim is to decrease the amount of Bti used in control programmes so that unwelcome impacts to the environment are further mitigated.

Literature cited Chapter I

Barnard, R. 2003. West nile disease and its control. Pesticide Outlook 14 (2): 76-79.

Becker, N., D. Petrić, M. Zgomba, C. Boase, C. Dahl, J. Lane, & A. Kaiser. 2003. *Mosquitoes and their control*. Kluwert Academic/Plenum Publishers, New York, N.Y., USA.

Blaustein, L., M. Kiflawi, A. Eitam, M. Mangel, & J.E. Cohen. 2004. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologia 138: 300-305.

Boisvert, M. 2005. Utilization of Bacillus thuringiensis var. israelensis (Bti)-based formulations for the biological control of mosquitoes in Canada. 6th Pacific

Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact. J.-C. Côté, I. S. Otvos, I.S., J.-L. Schwartz & C. Vincent. Victoria, BC.

Boisvert, M. & J. Boisvert. 2000. Effect of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. Biocontrol Sci Technol 10: 517-561.

Clements, H.W. & M.C. Newman. 2002. *Community ecotoxicology*. John Wiley & Sons, West Sussex, UK.

Duquesne, S., I. Kroeger, M. Kutyniok, & M. Liess. 2010. The potential of Cladocera as controphic competitors of the mosquito *Culex pipiens*. J. Med. Entomol. (in press).

Eitam, A., L. Blaustein, & M. Mangel. 2002. Effects of *Anisops sardea* (Hemiptera : Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. Hydrobiologia 485: 183-189.

Fleeger, J.W., K.R. Carman, & R.M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. Sci Total Environ 317: 207-233.

Hemingway, J., B.J. Beaty, M. Rowland, T.W. Scott, & B.L. Sharp. 2006. The innovative vector control consortium: improved control of mosquito-borne diseases. Parasitol today 22 (7): 308-312.

Killeen, G.F., U. Fillinger, & B.G.J. Knols. 2002. Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malar J 1: 8. doi:10.1186/1475-2875-1-8.

Marten, G.G. & J.W. Reid. 2007. Cyclopoid copepods. J Am Mosq Control Assoc 23: 65-92.

McMeniman, C.J., G.L. Hughes, & L. O'Neill Scott. 2011. A Wolbachia symbiont in *Aedes aegypti* disrupts mosquito egg development to a greater extent when mosquitoes feed on nonhuman versus human blood. 48(1): 76-84.

Mendis, K., A. Rietveld, M. Warsame, A. Bosman, B. Greenwood, & W.H. Wernsdorfer. 2009. From malaria control to eradication: the WHO perspective. Trop Med Int Health 14 (7): 802-809.

Mokany, A. & R. Shine. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecology 28: 33–37.

Mortimer, M.R. & H.F. Chapman. 1995. Acute toxic effects of (S)-methoprene and temephos to some Australian non-target aquatic crustacean species. Aust J Ecotoxicol 1: 107-111.

OECD. 2000. Guidance document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and safety. Paris, France.

Poulin, B., G. Lefebvre, & L. Paz. 2010. Red flag for green spray: adverse trophic effects of Bti on breeding birds. J Appl Ecol 47: 884-889.

Ramirez, J.L., L.S. Garver, & G. Dimopoulos. 2009. Challenges and Approaches for mosquito targeted malaria control. Curr Mol Med 9: 116-130.

Reiter, P. 2010. Yellow fever and dengue: a threat to Europe? Euro Surveill 15 (10): pii=19509. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?Articled=19509.

Ross, R. 1897. On some peculiar pigmented cells found in two mosquitos fed on malarial blood. Br Med J 2 (1929): 1786-1788.

Sachs, J., & P. Malaney. 2002. The economic and social burden of Malaria. Nature 415: 680-685.

Stav, G., L. Blaustein and J. Margalit. 2000. Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta Longiareolata* (Diptera:Culicidae) and community structure in temporary pools. J Vector Ecol 25: 190-202.

Takken, W., C. Constantini, G. Dolo, A. Hassanali, N'Fale Sagnon, & E. Osir. 2006. Mosquito mating behaviour. In B.G.J. Knols & C. Louis (Eds.), *Bridging laboratory and field research for genetic control of disease vectors*. Springer, Dordrecht, The Netherlands.

Thiboutot, M.M., S. Kannan, O.U.Kawalekar, D.J. Shedlock, A.S. Khan, G. Sarangan, P. Srikanth, D.B. Weiner, & K. Muthumani. 2010. Chikungunya: a potentially emerging epidemic? PLoS Negl Trop Dis 4 (4): e623. doi:10.1371/journal.pntd.0000623.

Weissmann, G. 2008. Gore's fever and Dante's *Inferno*: Chikungunya reaches Ravenna. The FASEB Journal 22: 635-638.

WHO. 2010. Generic risk assessment model for insecticides used for larviciding. World Health Organization. Geneva, Switzerland.

WHO. 2008. World malaria report 2008. World Health Organization. Geneva, Switzerland.

WHO. 2005. World malaria report 2005. World Health Organization and UNICEF. Geneva, Switzerland.

Zhou, G., P Kohlhepp, D. Geiser, M.D.C. Frasquillo, L. Vazquez-Moreno, & J.J. Winzerling. 2007. Fate and blood meal iron in mosquitos. J Insect Physiol 53 (11): 1169-1178.

Chapter II

Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetland areas in Germany

Alvine Larissa MEYABEME ELONO[1], Matthias LIESS[1*], and Sabine DUQUESNE[1, 2]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of System Ecotoxicology, Permoserstrasse 15, D-04318 Leipzig, Germany

[2]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, D-04318 Leipzig, Germany

Journal of Vector Ecology, in press.

Abstract Abundances of mosquito larvae and associated invertebrate communities were assessed in 27 temporary ponds during spring season in wetland areas of Germany. Four genera of mosquitoes were identified: *Aedes, Anopheles, Culex,* and *Culiseta*. We focused our analyses on *Aedes* spp. because this genus was the most abundant (92% of total abundance) and frequently encountered mosquito (present in 65% of investigated sites). The abundance of *Aedes* spp. was negatively associated with the abundance of competitors for food, and to a lesser extent with those of intraguild predators and strict predators. The influence of these natural antagonists on larvae of *Aedes* was stronger in ponds with higher levels of dissolved oxygen (53 ± 4%) than in ponds with lower levels (16 ± 1%). The abundances of antagonists overall explained 42% of the variation in abundance of *Aedes* spp. at sites with higher levels of dissolved oxygen. Of this explained variation, competitors accounted for 34.7%, whereas the abundance of intraguild predators, and strict predators accounted for only 6.8 and 0.5%, respectively. Therefore, the promotion of competing species might be an appropriate ecological approach for the control of *Aedes* spp. in temporary ponds in these areas.

Keyword Index: temporary ponds, *Aedes* species, competitors, predators, antagonists, spring season.

INTRODUCTION

Numerous vector-borne diseases are transmitted to humans and animals by mosquitoes in both tropical and temperate regions. For instance, the transmission of malaria involves mosquitoes of *Anopheles* species, whereas the transmission of arbovirus infections and filariasis mostly involves species of *Aedes* and *Culex* (Becker et al. 2003). Owing to the importance of mosquitoes as vectors for diseases in terms of public health, the ecological and environmental conditions that influence the abundances of these species are of great interest (Chaves and Koenraadt 2010).

A wide variety of aquatic environments (e.g. marshes, ponds, wells, drainage channels, lakes, and rivers) serve as breeding sites for mosquito larvae (Becker et al. 2003). However, many other invertebrate taxa (e.g. Crustacea, Acaria, insect larvae) share the same habitats (Campo et al. 2004, Bambaradeniya et al. 2004) and interact with mosquito larvae through competition and predation.

The term “competitors” in regard to mosquitoes refers to invertebrates that feed upon the same functional food as mosquito larvae (e.g. algae, bacteria, detritus, and protozoa, as reported by Mokany and Shine 2003b). Such competitors include, for example, Cladocera, Calanoida, and Harpacticoida (Knoechel and Holtby 1986, Dole-Oliver et al. 2000). The term “predators” refers to invertebrates that feed upon mosquito larvae, such as larvae of Odonata, Hydrophildae, and Dytiscidae (Becker et al. 2003, Kumar and Hwang 2006). Cyclopoida are considered here to be “intraguild predators”, in contrast to “strict predators”, because of their ability to feed on both algae (Thorp and Covich 1991) and mosquito larvae (Marten and Reid, 2007).

By either competing for the same food resources or preying on mosquito larvae, competitors and predators can reduce the survival of mosquitoes. Moreover, the presence of such antagonist species in a potential breeding site can deter gravid female mosquitoes from laying eggs (Stav et al. 2000, Eitam et al. 2002, Mokany and Shine 2003a, Blaustein et al. 2004, Duquesne et al. in press). The negative effects of several taxa on larval populations of mosquitoes have been demonstrated by laboratory and field studies (Knight et al. 2004, Kumar and Hwang 2006, Blaustein and Chase 2007, Banerjee et al 2009). Some of these studies focused on strict predators such as insect larvae (e.g. Odonata and Notonectidae; Stav et al. 2005, Saha et al 2010) and fish (Chandra et al. 2008), whereas others targeted intraguild predators such as Cyclopoida species (Marten and Reid 2007). Other studies focused on competition between larvae of different species of mosquito (Lounibos 2002, Juliano and Lounibos 2005, Juliano 2009). However, there have been few studies competition between mosquitoes and non-mosquito taxa as reported by Blaustein and Chase (2007). The above mentioned effects suggest that competitors and predators might as serve effective biological agents for the control of mosquitoes.

In the investigation reported herein, we focused on the aquatic communities of typical mosquito breeding sites, i.e on mosquito larvae and accompanying invertebrates of temporary ponds within natural wetlands of Central Germany. The primary aim of the study was to determine the influence of antagonist species on larval populations of mosquitoes. We hypothesized that abundances of mosquito larvae are inversely related to the densities of associated invertebrates, that is, to potential antagonists. We investigated these associations at three locations in natural wetlands: 1) a flood plain of the middle Elbe (Rosslau), 2) a flood region (Spreewald), and 3) a

flood plain of the River Parthe (Leipzig). Abiotic parameters (i.e. temperature, dissolved oxygen, pH, turbidity, emergent vegetation cover, and water depth and surface area) were also assessed to investigate the influence of these parameters on the abundances of mosquito larvae and their antagonists, as well as on their relationships.

We also aimed to distinguish the respective effects of competitors and potential predators on mosquito larvae. The abundance of mosquito larvae is limited mostly by competitors in natural temporary ponds and by predators in permanent ponds (Chase and Knight 2003). However, some predator species are also adapted to temporary ponds (e.g. Cyclopoida, small Dytiscidae, Turbellaria; Brendonck et al. 2002, Kumar and Ramakrishna 2003, Becker et al. 2003) and therefore may also play a role in the regulation of larval populations of mosquitoes.

The outcomes of this work will be useful for the development of measures to control mosquitoes that involve the application of ecological methods in temperate wetland areas.

MATERIALS AND METHODS

Investigated sites

The study sites were located in wetlands in three different federal states of Central Germany in which mosquitoes are known to be prevalent: Rosslau (Saxony-Anhalt), Spreewald (Brandenburg), and Leipzig (Saxony). The sites in Rosslau (51° 52’ N, 12° 14’ W) were located in the floodplain of the river Elbe. The sites in Spreewald (51° 02’ N, 13° 53’W) were located in flood areas in a region of traditional

irrigated agriculture that contained more than 200 small channels within an area of 484 km^2. The sites in Leipzig (51° 20' N, 12° 21' W) were located in the flood plain of the river Parthe. Twenty-seven sites in total were investigated. The numbers of sites per location were eight, ten, and nine for Rosslau, Spreewald, and Leipzig, respectively.

Monitoring communities of aquatic invertebrates and mosquito larvae

The sampling was performed once a week in each study area (Rosslau, Spreewald, and Leipzig) during the spring of 2007 (April 11th – June 13rd). Five subsamples of one to three liters (depending on the pond size and the load of suspended matter in the water) were collected at different points (with and without vegetation) in each pond, pooled into one sample, and filtered through a 55-µm mesh (Turner and Trexler 1997). The filtrate was conserved in 200 ml of distilled water and transported in plastic flasks to the laboratory to determine the taxa that were present. Micro-invertebrates (<5 mm in length) were identified and counted before further treatment to avoid the distortion of the shape of the ciliates and some rotifers by exposure to ethanol, which was used as a conservative agent (ethanol). Macro-invertebrates (>5 mm in length) were conserved with a mixture of ethanol:distilled water (70:30). For identification and quantification, an SMZ-645 stereo microscope (Nikon, Tokyo, Japan) at 50× magnification and an Axiostar Plus microscope (Zeiss, Düsseldorf, Germany) at 400× magnification were used. The following identification keys were used: Ward and Whipple (1959), Durand and Lévêque (1980), Schwab (1995), Narchuk and Glukhova (1999), Becker et al. (2003), Tachet et al. (2003), and Streble and Krauter (2006).

Abiotic parameters

Abiotic parameters were measured between 9 am and 11 am on each day that the mosquito larvae and invertebrates were sampled. The percentage of emergent vegetation cover and the surface area of the water were estimated visually; water temperature and pH were determined with an electronic pH meter (HANNA, Woonsocket, USA); dissolved oxygen (DO) with an electronic oxymeter (ExStik DO600, Extech, Walthman, USA) and turbidity with a turbidity meter (Turbiquant 1100 IR, Merck, Darmstadt, Germany). The depth of the water was assessed with a ruler, and the mean value of two to five random measurements (depending on the size of the pond) taken at different points in each pond was used.

Sample treatment

All the sites that were investigated were temporary ponds that dried out at least once during the period of the study. Samples that were collected from the same site before and after it had dried out were considered to be independent samples (IS). This is because in general (i) the abundance of mosquito larvae and the size of the ponds varied before and after drying out (Table 1) and (ii) these two variables, abundance of mosquito larvae and size of ponds, were not correlated. As a consequence, we collected a total of 77 independent samples from the 27 sites that were investigated. The numbers of independent samples per location and per site are shown in Table 1.

Table 1. The abundance of mosquito larvae and water surface area (size) for independent samples (IS) from sites at Rosslau, Spreewald, and Leipzig.

Location	Site	IS per site*	Mosquito larvae/L	Size (m^2)
Rosslau	I	a	38	400
		b	0.8	500
	II	a	0.4	300
		b	14	800
		c	0.4	100
	III	a	10.6	2
		b	5	50
		c	0	14
	IV	a	4	150
		b	0	1000
	V	a	13.4	30
		b	0.2	300
		c	0	8
	VI	a	15.8	15
		b	0.6	1000
		c	0	120
	VII	a	0.8	5
		b	1	1000
		c	0	50
	VIII	a	22.4	500
		b	0	1000
Leipzig	I	a	0	400
		b	5	1000
	II	a	6.2	300
		b	12	1000
		c	0	40
	III	a	8.6	100
		b	0	300
		c	19.2	1000
	IV	a	7	4
		b	13.8	70
		c	3.6	1000
	V	a	2.4	700
		b	20	30
		c	5	1000
	VI	a	0.6	700
		b	1.2	1000
		c	0.4	1000
	VII	a	8.6	200
		b	0	1000
		c	0	5
	VIII	a	0	4
		b	0	300
		c	4.4	1000
	IX	a	0	800
		b	6.2	1000

Location	Site	IS per site*	Mosquito larvae/L	Size (m^2)
Spreewald	I	a	0.6	1000
		b	1	100
		c	54.2	150
	II	a	2.4	120
		b	0	1000
		c	196	10
	III	a	7.6	70
		b	0.2	100
		c	28.2	1000
	IV	a	3.6	200
		b	1.2	800
		c	3.6	2
		d	0	50
	V	a	5	500
		b	0.8	300
		c	5.4	20
	VI	a	5.2	500
		b	1	150
		c	0.8	500
	VII	a	0	500
		b	1.4	50
		c	0.6	600
	VIII	a	0	800
		b	0	500
	IX	a	6.8	100
		b	12.4	500
		c	0	150
	X	a	0	300
		b	0.2	400
		c	0.2	50
		d	0	2

* When a pond dried out, sample taken before and after the drying out were classified as IS. Such samples are represented in the table by a, b, c, and d. IS were considered separately for statistical analyses because in general the abundance of mosquito larvae and the size of the pond varied before and after drying out and the two variables were not correlated.

Data analysis

The datasets at the sites of Rosslau, Spreewald, and Leipzig were analyzed both separately and in combination. The results from data on specific locations confirmed those on combined locations. Hence, only results obtained from combined data are shown in this paper (unless otherwise indicated). Data from independent samples (as explained above) were utilized for statistical analyses. They were subjected to log (x +1) transformation prior to all analyses.

Analysis of variance (ANOVA) was used to examine differences in the abundances of mosquito larvae between Rosslau, Spreewald, and Leipzig. Principal component analysis (PCA) was used to highlight the relationships between taxa that were identified during the present study. PCA is a linear unconstrained multivariate ordination method that is appropriate for describing variations in complex systems that are characterised by many species (Leps and Smilauer 2003). We chose a linear method because the length of the gradient determined by preliminary detrended correspondence analysis (DCA) was short (i.e. 2.005). The associations that were detected in the PCA between mosquito larvae and antagonists were tested for significance using the hierarchical model of multiple linear regressions. The hierarchical model with change statistics was used to assess the partial contribution of competitors, intraguild predators, and strict predators to the predictive capacity of antagonists with respect to the abundance of larvae of *Aedes* spp. Competitors, Cyclopoida, and predators were entered as first, second, and third blocks in this analysis, respectively. This order represents the increasing importance of the respective antagonists in temporary ponds (Chase and Knight 2003). The variance that is

explained by each additional group of antagonists (R^2 change and the corresponding F test) is given at each stage of the regressions.

The influence of the abiotic parameters on the species abundance of mosquito larvae and antagonists was determined using redundancy analysis (RDA), which is a constrained form of PCA (Leps and Smilauer 2003). In addition and using a t-test, we compared abiotic parameters between sites that simultaneously contained low abundances of mosquito larvae and antagonists and the other sites. This made it possible to check whether the abiotic parameters could explain the low occurrence of both mosquito larvae and antagonists (i.e. competitors and predators) in some study sites.

Outliers were detected using the Grubbs' test which detects one outlier at a time in a univariate dataset. The outlier identified is expunged and the test is iterated until the results show no outliers in the dataset. The Grubb's test uses the procedure of the extreme studentized deviate method. The ratio Z, which is the which is the ratio of the difference between the extreme value under analysis and the mean to the standard deviation (SD) from all values, including the extreme one, is calculated using the formula: $Z = \frac{|mean - value|}{SD}$ (GraphPad Software, Inc. 2005). Critical values for Z are given according to sample sizes in an extra table in the software. If the calculated value of Z is greater than the critical value for the sample size, the P value is less than 0.05 and the extreme value is considered to be an outlier.

The Grubb's test was carried out using the GraphPad QuickCalcs (http://www.graphpad.com/quickcalcs/Grubbs1.cfm; GraphPad Software, Inc. 2005), which is a free online calculator for scientits. PCA and RDA were performed using the program CANOCO for Windows 4.52 (ter Braak and Smilauer 2003). ANOVA, t-

tests, and linear regressions (95% CI) were performed using PASW Statistics (SPSS Inc. 2009).

RESULTS

Mosquito larvae

Mosquito larvae were found in 71% of the independent samples studied. Four genera of mosquito were identified: *Aedes, Anopheles, Culex,* and *Culiseta*. *Aedes* spp. was the most abundant type of mosquito collected (92% of the total numbers of larvae) and the most frequently encountered (present in 65% of the total number of sites).

Table 2. Abundances (mean ± SE (median)) and frequencies of mosquito larvae and invertebrate taxa for all samples (n = 77) collected at the three locations of Rosslau, Spreewald, and Leipzig.

Taxon	Abundance (individual /L)	Frequency (%)
Larva *Aedes* spp.	7 ± 3 (0.6)	65
Larvae of other mosquitoes	0.6 ± 0.2 (0)	22
Ciliata	63 ± 44 (0)	22
Rotifera	35 ± 8 (13)	51
Isopoda	7 ± 2	34
Ostracoda	488 ± 104 (104)	82
Cladocera	302 ± 58 (78)	78
Copepoda	362 ± 45 (260)	100
Larva Diptera[a]	8 ± 2 (0.6)	62
Larva Dytiscidae	0.2 ± 0.05 (0)	27
Larva Hydrophilidae	0.1 ± 0.02 (0)	18
Larva Scirtidae	0.1 ± 0.05 (0)	20
Larva Odonata	0.1 ± 0.03 (0)	14
Annelida	1 ± 0.5 (0)	7
Nematoda	3 ± 1 (0)	14
Planaria	7 ± 2 (0)	31

[a]Larvae of Diptera other than mosquitoes

The other genera of mosquitoes (i.e. *Anopheles, Culex,* and *Culiseta*), which represented the remaining 8% of the total number of larvae, were present in 22% of the sites (Table 2). The mean abundances of mosquito larvae showed no significant difference between the Rosslau, Spreewald, and Leipzig regions (the means and standard errors (medians) were 6 ± 2 (1), 11 ± 6 (1), and 5 ± 1 (4) individuals per liter, respectively; data not shown; ANOVA, $P > 0.05$). Owing to the fact that *Aedes* spp. was the dominant mosquito species at the study sites (Table 2), we focused our analyses on these mosquitoes. Whenever the mosquito species that were characterized by low abundances were considered, they were classified as the single group "other mosquitoes".

Abundances and distribution of associated invertebrate taxa

The associated invertebrate taxa that were identified included Ciliata, Rotifera, Microcrustacea (i.e. Cladocera, Copepoda, and Ostracoda), Isopoda (i.e *Asellus*), Annelida, Nematoda, Planaria, and larvae of the insects Dytiscidae, Hydrophilidae, Scirtidae (i.e. *Hydrocyphon*), Diptera (i.e. mosquitoes, *Dixa*, *Mochlonyx*, Chironomidae, Chaoboridae, and Stratiomyidae), and Odonata (i.e. Anisoptera and Zygoptera). Microcrustacea were the most abundant and the most frequently encountered invertebrate taxon among the samples (Table 2). Insects other than mosquitoes, with the exception of Diptera, were the least abundant. The abundances and frequencies of all invertebrate taxa among the sites are shown in Table 2.

Influence of associated invertebrate taxa on *Aedes* larvae

The results of the principal component analysis (PCA) showed that the abundance of larvae of *Aedes* spp. was associated negatively with the abundance of antagonists and more specifically of (a) some competing taxa (i.e. *Ceriodaphnia, Chydorus, Daphnia, Simocephalus*, Calanoida, and larvae of Chironomidae), (b) some strict predatory taxa (i.e. insect larvae of the groups *Chaoborus*, Dytiscidae, Hydrophilidae, Anisoptera and Zygoptera), and (c) intraguild predators (i.e. Cyclopoida species) (Fig. 1).

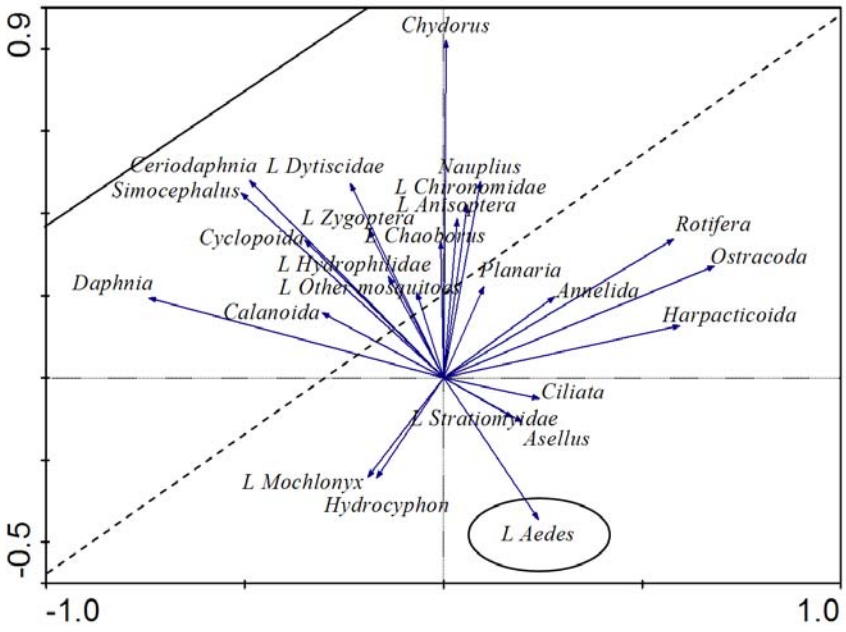


Figure 1. Ordination plot for principal component analysis (PCA) performed on all data (n = 77). This plot shows the different associations between the abundances of members of taxa that were identified in the present study. Arrows between the solid and the dashed oblique lines show taxa that were negatively associated with larvae of *Aedes* spp. L: larvae.

Larvae of *Aedes* spp. were also associated negatively with the abundances of larvae of other mosquitoes (Fig. 1). Slight and positive associations were found

between the abundance of larvae of *Aedes* spp. and those of other potential competitors (e.g. Ostracoda, Harpacticoida, Annelida, and the Scirtidae *Hydrocyphon*) or predators (e.g. larvae of the insects *Mochlonyx*) (Fig. 1).

Interactions between associated invertebrate taxa and *Aedes* spp. in relation to the abiotic parameters

In a first analysis, RDA was carried out on data for all sites to test for the influence of abiotic parameters on the abundances of mosquito larvae and associated invertebrate communities. The results showed that larvae of *Aedes* spp. and their antagonists (identified in Fig. 1) did not correlate significantly with the abiotic parameters that were investigated (i.e. temperature, dissolved oxygen (DO), pH, turbidity, emergent vegetation cover, and water depth and surface area; data not shown). Only Ostracoda which had a slightly positive association with *Aedes* spp. (Fig. 1), showed significant correlations with turbidity, emergent vegetation cover, pH, and water surface area (data not shown). The means (medians) of these parameters, for all sites, were as follows: temperature, 15.2 ± 0.4°C (14°C); DO, 32 ± 3% (27%); pH, 7.06 ± 0.08 (7.2); turbidity, 18 ± 3 NTU (8.3 NTU); depth, 0.12 ± 0.07 m (0.11 m); water surface area, 400 ± 40 m^2 (300 m^2); and emergent vegetation cover, 50 ± 4% (70%).

In a second analysis, we divided the dataset into two categories: (1) sites with lower abundances of both *Aedes* larvae and antagonists (< 1 larva/L and < 100 antagonists/L); these sites had low levels of DO (16 ± 1%) (n = 42), and (2) sites with higher abundances of organisms (i.e. *Aedes* larvae and antagonists) which had higher levels of DO (53 ± 4%) (n = 35). The difference in DO between these two categories

was significant (t_{75} assuming equal variances, P = 0.018). The thresholds of 1 larvae/L and 100 antagonists/L were selected, as these values, were close to the medians for larvae of *Aedes* spp. (0.6 individual/L) and antagonists (89 individuals/L) over all sites. The sites with high levels of DO contained approximately 65% more antagonists than the sites with low DO (medians of 127 and 77 individuals/L, respectively). We used linear regressions to test for the significance of the associations that we found between larvae of *Aedes* spp., other mosquito larvae, and antagonists (Fig. 1). This analysis was carried out for both categories (sites with higher and sites with lower DO) and the results obtained are detailed below.

- **At sites with higher levels of DO**

In this subset (n = 35), one sample was found to be an outlier (Fig 2A) by the Grubb's test (P < 0.05). The results that were obtained both including and excluding the outlier are presented in this section because of the substantial influence of this single outlier on the analyses.

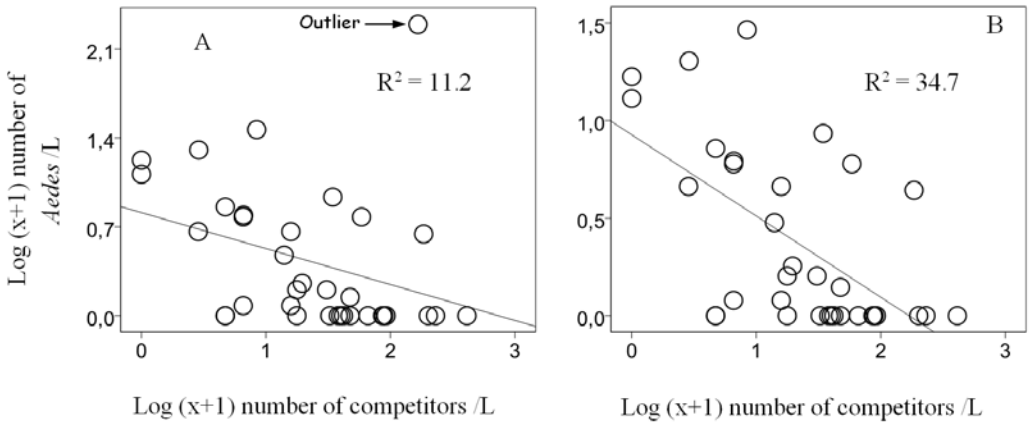


Figure 2. Relationships between *Aedes* spp. and competitors obtained with data for ponds with high levels of dissolved oxygen. A and B show the scatter plots obtained for inclusion and exclusion of the outlier, respectively.

When the outlier was not considered, the abundance of the pooled antagonists explained 42% of the variation observed in the abundance of larvae of *Aedes* spp (100 x $\sum R^2$ change, Table 3).

Table 3. Multiple linear regressions (hierarchical model) showing the negative relationships between the abundances of larvae of *Aedes* spp. and antagonists in sites with high levels of dissolved oxygen and low dissolved oxygen. The partial contribution (R^2 change) of competitors, intraguild predators and strict predators to the predictive capacity of antagonists with respect to the abundance of larvae of *Aedes* spp. is provided.

Subset	Model	Antagonists	R^2 change	F change	P change	Partial R
high DO	1	Competitors	0.347**	17.005	0.000	-0.589
-outlier	2	Competitors				-0.549
		Intraguild predators	0.068	3.590	0.067	-0.322
(n = 34)	3	Competitors				-0.510
		Intraguild predators				-0.314
		Strict predators	0.005	0.283	0.599	-0.097
high DO	1	Competitors	0.112*	4.162	0.049	-0.335
+outlier	2	Competitors				-0.276
		Intraguild predators	0.072	2.809	0.103	-0.284
(n = 35)	3	Competitors				-0.214
		Intraguild predators				-0.266
		Strict predators	0.039	1.552	0.222	-0.218
Low DO	1	Competitors	0.034	1.419	0.241	-0.185
(n = 42)	2	Competitors				-0.142
		Intraguild predators	0.014	0.559	0.459	-0.119
	3	Competitors				-0.131
		Intraguild predators				-0.109
		Strict predators	0.006	0.260	0.613	-0.083

* and ** indicate significance at levels of 0.05 and 0.001, respectively.

With respect to the explained variation, the abundance of the competitors accounted for 34.7% (Fig. 2B), whereas the abundances of the intraguild and strict predators accounted for 6.8 and 0.5%, respectively (Table 3). The influence of the other mosquitoes on the effects of the antagonists was not significant ($P > 0.05$) and accounted for only 0.8% (data not shown).

When the outlier was considered, the abundance of the pooled antagonists explained 22.4% of the variation observed in the abundance of larvae of *Aedes* spp. (Table 3). With respect to this explained variation, the competitors, intraguild predators, and strict predators accounted for 11.2, 7.2, and 3.9%, respectively (Table 3). The influence of the other mosquitoes on the effects of the antagonists was not significant ($P > 0.05$) and accounted for only 1.9% (data not shown).

Overall, the findings obtained for the sites with higher levels of DO ("with" and "without" the outlier, Fig. 2A & 2B, respectively) revealed that, among the groups of antagonists studied, the abundance of competitors was the main factor that affected the abundance of larvae of *Aedes* spp. (R^2 change, Table 3). The partial contributions of the abundance of intraguild and strict predators were small (R^2 change, Table 3) and were not significant (P change, Table 3).

Factors that could explain the difference between the outlier and the other observations were unclear. The difference could be related to parameters that were not investigated during the present study (e.g. the availability of food).

- **At sites with lower DO**

No outliers were identified in this subset (n = 42). At sites with lower levels of DO, the abundance of the antagonists explained only 5.4% of the variation observed

in the abundance of *Aedes* spp. (Table 3). Therefore, the negative influence of antagonists on larvae of *Aedes* spp. was small and not significant at these sites (Table 3).

DISCUSSION

Abundance of *Aedes* spp.

Aedes species were the most abundant species of mosquito recorded in this study, which was carried out during the spring of 2007 in wetlands of Rosslau, Leipzig, and Spreewald. This finding with respect to larval stages corresponds to that of Schäfer et al. (1997), who reported a predominance of *Aedes* species (i.e. *Ae.communis, Ae. rusticus, Ae. punctor, Ae. cantans,* and *Ae. dianteus*) among adult mosquitoes collected from late April to early June, 1993, in Bienwald (Germany). Therefore, *Aedes* species seem to be the predominant mosquitoes in Germany during spring. Members of this genus are involved potentially in the transmission of arbovirus infections and filariasis in Germany (Becker et al. 2003). Therefore, the control of *Aedes* species as potential vectors of disease is very important.

Influence of associated invertebrate taxa on larvae of *Aedes* spp.

Our results showed that the abundance of *Aedes* spp. was mainly negatively correlated with the abundance of food competitors. Therefore, when present in the same habitat, *Ceriodaphnia* spp., *Chydorus* spp., *Daphnia* spp., *Simocephalus* spp., Calanoida and larvae of Chironomidae compete efficiently for food resources and affect the abundance of *Aedes* spp. Similarly, Chase and Knight (2003) demonstrated that non-mosquito competitors (e.g. larvae of Chironomidae, Cladocera) limited the

abundance of *Anopheles quadrimaculatus* and *Culex pipiens* to a great extent in temporary ponds of Northwest Pennsylvania (USA). All the relevant species of competitors that we identified in our study are known to inhabit the littoral zones of ponds (Adamczuk 2006). Obviously, competition between these species and the mosquitoes that live mostly in shallow waters (Abdullah and Merdan 1995) is especially strong.

In addition, our results showed that predators also influenced, although to a lesser extent than competitors, the larval populations of *Aedes* spp in temporary ponds. Cyclopoida, which are intraguild predators of mosquito larvae, were found to act as antagonists in our study. This is in agreement with the results of a number of laboratory and field studies around the world that have shown that Cyclopoida prey upon mosquitoes (Marten and Reid 2007). Strict predators of mosquitoes such as Zygoptera and Dytiscidae exerted the smallest influence in our study, which might have been due to the relatively low abundances of these taxa in temporary ponds. Banerjee et al. (2009) found no larvae of *Aedes* species in habitats that contained strict predators such as beetles and Odonata. The negative relationship reported by these authors might be a consequence of the fact that the investigated ponds had been in existence for long periods; indeed, such biotopes shelter abundant predators, as mentioned by Schneider (1997).

Influence of the abiotic parameters

The abiotic parameters investigated, with exception of dissolved oxygen (DO), (i.e. temperature, pH, turbidity, emergent vegetation cover, and water depth and surface area) did not influence the abundance of the antagonists identified in the

present study. Given that the investigation took place during one season (i.e. spring), the restricted variations in abiotic parameters between sites may not have been sufficient to elicit differences in species abundances.

Concerning larvae of *Aedes* spp., the results support the hypothesis that mosquito larvae, including *Aedes* spp., are not particularly sensitive to variations in the water-quality parameters measured here. For example, in a laboratory study, the survival of larvae of *Aedes aegypti* was not affected by pH values that ranged from 4 to 11 (Clark et al. 2004). Similarly, in a field study, water-quality parameters were found to be of minor importance for the abundance of mosquito larvae (Beketov et al. 2010).

However, the results of the study reported herein showed that the sites with simultaneously lower abundances of *Aedes* spp. and antagonists differed from the sites with higher abundances of these organisms in terms of the level of DO and that, the interactions that were detected between the two groups differed. In fact, the negative correlation between antagonists and larvae of *Aedes* spp. was much stronger in water with high levels of DO than in water with low DO. Therefore, the influence of antagonists on the abundance of *Aedes* spp. might be particularly strong in sites with high concentrations of DO. The present study highlights the importance of the indirect effect of an environmental parameter on interactions between mosquito larvae and their associated communities.

CONCLUSION

The results reported herein showed that invertebrate species that are antagonists to mosquito larvae limit the larval populations of *Aedes* spp. during spring and thus reduce the suitability of temporary ponds as mosquito breeding sites in wetland areas in European temperate regions. Among these groups of antagonists, the food competitors for food influenced the larval populations more strongly than predators. Therefore, competitors might have potential as biological agents for use in the control of the abundance of larvae of *Aedes* spp. Such findings with regards to the ecological conditions related to biological interactions between mosquito larvae and associated species are important for the implementation of appropriate control measures and integrated management of areas infested with mosquitoes.

ACKNOWLEDGMENTS

We are grateful to Mikhail Beketov and Kaarina Foit for help with the statistical analyses. We thank the anonymous reviewers whose comments and suggestions improved the present manuscript greatly. This study was funded by the DAAD (German Academic Exchange Service) and the Helmholtz Centre for Environmental Research – UFZ Leipzig, Germany.

REFERENCES CITED

Abdullah, M.A.R. and A.I. Merdan. 1995. Distribution and ecology of the mosquito fauna in the southern Saudi Arabia. J. Egypt. Soc. Parasitol. 25 (3): 815-837.

Adamczuk, M. 2006. Effect of biotic zones on species distribution of Cladocera in mesotrophic Lake Piaseczno. Acta Agrophysica 7 (2): 281-288.

Bambaradeniya, C.N.B., J.P. Edirisinghe, D.N. De Silva, C.V.S. Gunatilleke, K.B. Ranawana, and S. Wijekoon. 2004. Biodiversity associated with an irrigated rice agro-ecosystem in Sri Lanka. Biodiversity and Conservation 13: 1715-1753.

Banerjee, S., G. Aditya, N. Saha, and G.K. Saha. 2009. An assessment of macroinvertebrate assemblages in mosquito larval habitats-space and diversity relationship. Environ. Monit. Assess. DOI 10.1007/s10661-009-1137-9

Becker, N., D. Petrić, M. Zgomba, C. Boase, C. Dahl, J. Lane, and A. Kaiser. 2003. *Mosquitoes and their control*. Kluwert Academic/Plenum Publishers, New York, N.Y., USA.

Beketov, M.A., Y.A.Yurchenko, O.E. Belevich, and M. Liess. 2010. What environmental factors are important determinants of structure, species richness and abundance of mosquito assemblages. J. Med. Entomol. 47(2): 129-139.

Blaustein, L., M. Kiflawi, A. Eitam, M. Mangel, and J.E. Cohen. 2004. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologia 138: 300-305.

Blaustein, L., and J.M. Chase. 2007. Interactions between mosquito larvae and species that share the same trophic level. Annual. Rev. Entomol. 52: 489-507.

Brendonck, L., E. Michels, L. De Meester, and B. Riddoch. 2002. Temporary pools are not “enemy-free”. Hydrobiologia 486: 147-159.

Campos, R.E., L.A. Fernandez, and V.E. Sy. 2004. Study of the insects associated with the floowater mosquito *Ochlerotatus albifasciatus* (Diptera : Culicidae) and their possible predators in Buenos Aires Province, Argentina. Hydrobiologia 524: 91-102.

Chandra, G., I. Bhattacharjee, S.N. Chatterjee, and A. Ghosh. 2008. Mosquito control by larvivorous fish. Indian J. Med. Res. 127: 13-27.

Chase, J.M., and T.M. Knight. 2003. Drought induced mosquito outbreaks in wetlands. Ecol. Lett. 6: 1017-1024.

Chaves, L.F. and C.J.M.. Koenraadt. 2010. Climate change and highland malaria: fresh air for a hot debate. Q. Rev. Biol. 85 (1): 27-55.

Clark, T.M., B.J. Flis, and S.R.Remold. 2004. pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae. J. Exp. Biol. 207: 2297-2304.

Dole-Oliver, M.-J., D.M.P. Galassi, P. Marmonier, and M. Creuzé des Châteliers. 2000. The biology and ecology of lotic microcrustaceans. Freshwater Biol. 44 : 63-91.

Duquesne, S., I. Kroeger, M. Kutyniok, and M. Liess. 2010. The potential of Cladocera as controphic competitors of the mosquito *Culex pipiens*. J. Med. Entomol. (in press).

Durand, J.R. and C. Lévêque. 1980. *Aquatic flora and fauna of Sahelo-Sudanese Africa*, Volume I, Office for Overseas Scientific and Technical Research (O.R.S.T.O.M), Paris, France.

Eitam, A., L. Blaustein, and M. Mangel. 2002. Effects of *Anisops sardea* (Hemiptera : Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. Hydrobiologia 485: 183-189.

GraphPad Software, Inc. (2005) *PRISM Version 4.0*. GraphPad Software Inc., San Diego, California, USA.

Juliano, S.A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. Annu. Rev. Entomol. 54: 37-56.

Juliano, S.A. and L.P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol. Lett. 8: 558-574.

Knight, T.M., J.M. Chase, C.W. Goss, and J.J. Knight. 2004. Effects of interspecific competition, predation and the interaction on survival and development time of immature *Anopheles quadrimaculatus*. J. Vector Ecol. 29(2): 277-284.

Knoechel, R. and L.B. Holtby. 1986. Cladoceran filtering rate: body length relationship for bacterial and large algal particles. Limnol. Oceanogr. 31(1): 195-200.

Kumar, R. and R.T. Ramakrishna. 2003. Predation on mosquito larvae by *Mesocyclops thermocyclopoides* (Copepoda: Cyclopoida) in the presence of alternate prey. Internat. Rev. Hydrobiol., 88(6): 570-581.

Kumar, R. and J.S. Hwang. 2006. Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zool. Stud. 45(4): 447-466.

Leps, J. and P. Smilauer. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, UK.

Lounibos, L.P. 2002. Invasions by insect vectors of human disease. Annu. Rev. Entomol 47: 233-266.

Marten, G.G. and J.W. Reid. 2007. Cyclopoid copepods. J. Am. Mosq. Control Assoc.23: 65-92.

Mokany, A. and R. Shine. 2003a. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral. Ecology 28: 33–37.

Mokany, A. and R. Shine. 2003b. Competition between tadpoles and mosquito larvae. Oecologia 135: 615–620.

Narchuk, E.P. and V.M. Glukhova 1999. Higher insects, Diptera, pp. 137–151. In S.J. Tsalolikhin (eds), Culicidae: key to the freshwater invertebrates of Russia and the adjacent lands, Vol. 4. Zool. Inst. Russ. Acad. Sci., St Petersburg, Russia. [in Russian]

Saha, N., G. Aditya, G.K. Saha, and S.E. Hampton. 2010. Opportunistic foraging by heteropteran mosquito predators. Aquat. Ecol. 44: 167-176.

Schäfer, M., V. Storch, A. Kaiser, M. Beck, and N. Becker 1997. Dispersal behavior of adult snow melt mosquitoes in the upper Rhine valley, Germany. J. Vector Ecol. 22(1): 1-5.

Schwab, H. (1995).). *Freshwater animal*. 1st ed. Klett Schubuch, Stuttgart, Germany.

Schneider, D.W. 1997. Predation and food web structure along a habitat duration gradient. Oecologia 110: 567-575.

SPSS Inc. (2009) *PASW Statistics 17.0.3 for Window*. SPSS Inc., Chicago, Illinois.

Stav, G., L. Blaustein and J. Margalit. 2000. Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta Longiareolata*

(Diptera:Culicidae) and community structure in temporary pools. J. Vector Ecol. 25: 190-202.

Stav, G., L. Blaustein, and J. Margalit. 2005. Individual and interactive effects of predator and controphic species on mosquito populations. Ecol. Appl. 15 (2): 587-598.

Streble, H., and D. Krauter. 2006. *The life in water, Microflora und Microfauna of freshwaters*. 10th ed. Franckh-Kosmos, Stuttgart, Germany.

Tachet, H., P. Richoux, M. Bournaud, and P. Usseglio-Polatera. 2003. *Freshwater invetebrates, systematic, biology, ecology*. CNRS, Paris, France.

ter Braak, C. J .F. and P. Smilauer. 2003. *Canoco for Windows Version 4.52*. Biometrics-Plant Research International. Wageningen, The Netherlands.

Thorp, J. H. and A.P. Covich. 1991. *Ecology and classification of North America freshwater invertebrates*. Academic Press, Inc. San Diego, California, USA.

Turner, A.M. and J.C. Trexler. 1997. Sampling aquatic invertebrates from marshes: evaluating the options. J. N. Am. Benthol. Soc. 16(3): 694-709.

Ward, H.B, and G.C. Whipple. 1959. Fresh-water biology, 2nd ed. John Wiley & Sons, Inc. New York & London, USA.

Chapter III

Population response of *Culex pipiens* to thiacloprid is altered by interspecific interactions

Alvine Larissa MEYABEME ELONO[1], Sabine DUQUESNE[2], Kaarina FOIT[1], Matthias LIESS[1]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of System Ecotoxicology, Permoserstrasse 15, D-04318 Leipzig, Germany

[2]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, D-04318 Leipzig, Germany

Ecotoxicology, in preparation for submission.

Abstract We assessed ecological effects of the insecticide thiacloprid on larval populations of *Culex pipiens* and their associated invertebrate taxa in an outdoor microcosm investigation lasting 53 days. The experimental design crossed the presence or absence of a community with three concentrations: 0.1 µg/L, 1 µg/L, and 10 µg/L. The addition of invertebrate community significantly reduced the abundance of *Cx. pipiens* larvae at all concentrations levels. Exposure to thiacloprid showed no effects at concentrations lower than 10 µg/L. After the second exposure to 10 µg/L, populations of *Cx. pipiens* showed contrasting effects in the presence and absence of added community. In the "no community added" microcosms, the larval abundance of *Cx. pipiens* decreased temporarily whereas in the setup "community added", a temporary increase was observed. This difference in effects is likely explained explained by interspecific interactions. Increase of larval *Cx. pipiens* in the treatment 10 µg/L of the "community added" setup was due evidently to the elimination of some competitors for food sources (Ostracoda, Copepoda, and larvae of Chironomidae) and predators (Copepoda) of mosquito larvae. Besides in this setup, the recovery potential of invertebrate communities following thiacloprid stress depended obviously on the presence of adults outside the water. The present results are useful for predicting the effects at community level of toxicants in general and of larvicides in particular.

Keywords: Thiacloprid · *Culex pipiens* · invertebrate taxa · competitors · predators · indirect effects

INTRODUCTION

Mosquitoes serve as vectors for the transmission of dangerous medical pathogens and parasites such as viruses, protozoans, and nematodes to humans and animals (Becker et al 2003). These parasites and pathogens are etiological agents of serious diseases (e.g. malaria, dengue fever, yellow fever, encephalitis, filariasis) that are responsible for the disability and the death of millions of people in the world every year (WHO 2005; Reiter 2010).

Since the first discovery of a disease borne pathogen in mosquitoes (Ross 1897), numerous control measures, including various insecticides, have been exploited in control programmes worldwide (Barnard 2003; Ramirez et al. 2009; Reiter 2010). However, the number of insecticides currently available in the market is not sufficient to face the delivery from mosquitoes (Hemingway et al 2006). Therefore, the great need of new insecticides with a new mode of action was recently raised as an urgent tool for mosquito control (Hemingway et al 2006; Mendis et al. 2009).

Thiacloprid is a new neonicotinoid insecticide which is selectively active against insects (Schmuck 2001; Tomizawa et al. 2007). It acts by inducing the disruption of the nervous system of insects through inhibition of nicotinic acetylcholine receptors (USEPA 2003). Recently in a laboratory experiment, Beketov and Liess (2008) showed that mosquito larvae and especially larvae of *Culex pipiens* are highly sensitive to the insecticide thiacloprid.

The present study aimed at investigating ecological risks and efficiency of thiacloprid for mosquito control in aquatic environments. Specifically, we assessed the ecological effects of threshold levels of thiacloprid appropriate for the control of larval

populations of mosquitoes in an outdoor microcosm investigation. The study was conducted in the area of the Helmholtz Centre for Environmental research –UFZ, Leipzig (Germany). The knowledge acquired may be useful to support and improve the risk assessment of the potential new insecticides for mosquito control.

MATERIALS AND METHODS

Setup

We conducted an experiment with 32 outdoor freshwater microcosms during the summer 2007. The experimental design consisted of the presence or absence of an invertebrate community crossed with three concentrations of thiacloprid: 0.1, 1, and 10 µg/L. The pesticide was applied after 5, 19, and 34 days following their natural colonization by local populations of *Culex pipiens*. The abundance of oviposition and larvae of *Cx. pipiens* was assessed weekly and, the abundance of the community was monitored monthly. The experiment was terminated after 53 days.

Micrososms

The outdoor microcosm investigation took place from July 6th to August 27th 2007 in the area of the Helmholtz Centre for Environmental Research-UFZ, Leipzig (Germany). Each of the 32 freshwater microcosms was constituted in graduated plastic buckets of 90 litres with an opening of 0.5m. They were buried at 2/3 in the ground to simulate ponds in the natural environments. Each microcosm contained 2 cm mixed sediments from natural ponds of Rosslau, Leipzig and Spreewald (Central Germany).

Sediments were dried at 120°C in an oven (Heraeus Instruments, Germany) during 12 hours to minimize unwanted entering of organisms from sediments. The level of ponds was completed to 60 L with tap water.

The 32 microcosms were divided into two setups: "no community added" and "community added" microcosms. We introduced no organisms in microcosms of the "no community added" setup. We enriched the microcosms of the "community added" setup with invertebrate communities from natural ponds of Rosslau, Leipzig, and Spreewald. The invertebrate community was concentrated by filtering around 300 L of pond water to 1L through a 55 µm mesh. This procedure was repeated several times and the 1-litre subsamples were pooled together into a single sample. Each microcosm of the "community added" setup received 500 mL of this community concentrate. Taxa that were collected comprised Arcellinida, Collembola, Acaria, Ostracoda, Cladocera, Copepoda, Hydra, and larvae of insects (i.e. Chironomidae and Odonata). We started the food conditions of microcosms by providing batch-culture green algae (*Desmodesmus subspicatus*) with a food quantity of 3.1 x 10^4 cells/mL (0.13 mgC/L); algae obtained from the University of Göttingen, Germany. After the enrichment with invertebrate communities, all microcosms were covered with nets during five days to let the biocoenosis accommodate. One day before the first exposure of microcosms to thiacloprid, the mean (± SE) abundance of added organisms were 272 ± 54 and 0 individuals/L in "community added" and "no community added" microcosms, respectively.

Temperature (°C) was measured every two hours using µS-LOG540 data logger (Driessen + Kern, Bad Bramstedt, Germany) in two randomly selected microcosms of each setup. We measured the other abiotic parameters at least once

after each application of thiacloprid. Dissolved oxygen (DO, in % saturation) was measured *in situ* with an electronic oxymeter ExStik DO600 (Walthman, MA, USA). Electric conductivity (EC, µS/cm), pH, and total dissolved solids (TDS, mg/L) of water were measured *in situ* using a multimeter ExStik II EC500 (Waltham, MA, USA). Turbidity, which was expressed in Nephelometric turbidity units (NTU), was measured in the laboratory with a turbidity-meter Turbiquant 1100 IR Merck (Darmstadt, Germany). Before the measurement of turbidity, we removed large particles of the sample by filtering (mesh-size, 180 µm). The chlorophyll was measured in Relative fluorescence units (RFU) using a spectrofluorometer Spectramax Gemini EM (USA; wavelengths of 4 400 nm for excitation, 700 nm for emission and 690 nm as cutoff).

Exposure to thiacloprid

We exposed the microcosms at days 5, 19, and 34. The frequency of the last two applications depended on the recovery of larval population of *Cx. pipiens* in microcosms treated with effect concentrations. We used the suspension concentrate Calypso® 480 g/l of thiacloprid obtained from Agrar-Handel und Transport (Schafstädt, Germany). The setups “no community added” and “community added” were exposed to thiacloprid at the nominal concentrations of 0 (control), 0.1, 1, and 10 µg/L with four replicates for each concentration and setup. A stock solution of thiacloprid at 10 mg/L was prepared in brown graduated bottles (Calypso® 480 g/l in distilled water, total volume 2 L). Aliquots of 0.6 mL, 6 mL and 60 mL of this stock solution were spread on the surface of microcosms to attain the nominal concentrations

of 0.1µg/L, 1µg/L and 10µg/L, respectively. The water was then stirred gently in five turns using a 1-cm diameter stick.

The selected nominal concentrations were based on a 12-day and on a 5-day lethal median concentration (LC50) of thiacloprid of 6.04 µg/L (Beketov and Liess 2008) and 9.91 µg/L (unpublished data) for larval *Cx pipiens*, respectively. For the 5-day LC50, we exposed newly hatched larvae of *Cx. pipiens* (age: 24h) to the following thiacloprid concentrations: 0 (control), 0.3, 1, 3, 10, and 33 µg/L, with 10 replicates for each concentration level. Larvae were individually and continuously exposed to these concentrations in 80-mL flasks that contained 40 mL artificial Elendt M4. To simulate the environmental conditions of the freshwater microcosms, we added 2 mm of sediment per flask. No additional food source was provided during the exposure time to thiacloprid. The environmental conditions were maintained at a temperature of 20 ± 1°C and a photoperiod of 16:8 h (light:dark).

Validation of exposure concentrations

To quantify the actual thiacloprid concentrations in the microcosms over time, the microcosms were sampled always two hours after each application of thiacloprid, and at least two times between subsequent applications. A subsample of 500 mL of water was taken from each microcosm. The subsamples from the same exposure concentration were pooled to a single sample. Before measurements, samples were concentrated using solid phase extraction on Chromabond C18 Hydra (Macherey-Nagel, Düren, Germany). Analyses were performed with liquid chromatography (high-performance liquid chromatography system with Diodenarray Detector II Series 2000, binary pump, autosampler, column oven (30C), Perkin Elmer, Wellesley, MA,

USA). The injection volume was 100 µl, dissolved in 25% acetonitril/water solution with gradient-grade pump program. The limit of detection was 0.01 µg/L. The column LiChrospher 60, RP-select B, 5 µm (Merck, Darmstadt, Germany) was used for separation (Kommunale Wasserwerke Leipzig laboratory, Germany).

Monitoring of *Cx. pipiens* and invertebrate community

The oviposition and larval abundance of *Cx. pipiens* were recorded at least two times per week. A record was insured before and after each application of thiacloprid. Egg rafts were counted on the water surface of each microcosm. Mosquito larvae were sampled by dipping four times a volume of 250 mL at the edge of each microcosm (adapted standard dipping technique, WHO 1975). The four samples were averaged and the abundance was expressed in individual /dip. Counted larvae were immediately returned to the microcosm.

The invertebrate taxa associated with larvae of *Cx. pipiens* were recorded in general once before and once after each application of thiacloprid. To attain a representative sampling of the communities, we took 6 samples of 250 ml from different sides and depths of each microcosm. The subsamples were pooled to a single sample and gently stirred. One litre of the pooled sample was filtered through a 55 µm mesh and conserved in a 15 ml-flask with a mixture ethanol:distilled water (70:30). The remaining part of the pooled sample was put back to the respective pond. In the laboratory, taxa were identified and counted using a binocular Nikon SMZ 645 (Göttingen, Germany) and a microscope ZEISS Axiostar (Göttingen, Germany). The identification keys which were used were those of Ward and Whipple (1966), Durand

and Lévêque (1980), Schwab (1995), Narchuk and Tumanov (1999), Becker *et al*. (2003), Tachet *et al*. (2003) and Streble and Krauter (2006).

Data analyses

We assessed the differences in oviposition and larval abundances of *Cx. pipiens* between setups using a t-test, and between treatments (i.e. control, 0.1, 1, and 10 µg/L) within each setup using ANOVA. When a significant ANOVA (i.e. $P < 0.05$) was found, the Dunnett's post hoc test followed for the comparisons between controls and thiacloprid concentrations. MANOVA was used to compare chlorophyll and abiotic parameters between treatments. Prior to these analyses, data were subjected to Log (x+1) transformation (x represented the abundance value).

Principal component analysis (PCA) was used to analyse the relationships between *Cx. pipiens* and their invertebrate communities associated. PCA is a linear unconstrained multivariate ordination method that is appropriate to show associations between many species in a complex ecosystem (Leps and Smilauer 2003). We chose a linear method because the length of gradient was short (i.e. < 2). The associations detected in the PCA were tested for the significance using simple model of linear regressions.

The Principal Response Curves (PRC) was used to assess the responses of communities to thiacloprid treatments. PRC is a multivariate technique which is based on the Redundancy Analysis (RDA), the constrained form of the principal component analysis (Van den Brink and Ter Braak 1999). The result of PRC is a diagram showing the deviations over the time of treatments from the controls. In a different graph, the weights of the response variables (i.e. invertebrate taxa) are represented. The weight

indicates the affinity of the response variable with the PRC. Monte Carlo permutation tests were used to examine the statistical significance of the PRC for the entire time series in the RDA from which the PRC were derived (Leps and Smilauer 2003). This test was repeated for each sampling day to investigate the responses over the time. To down-weight high values, data used for PRC and RDA were subjected to ln (10x +1) transformation (Leps and Smilauer 2003).

ANOVA, Dunnett's test, and linear regressions were carried out in PASW version 17.0 (SPSS Inc., 2009). PRC and RDA, and PCA were carried out in CANOCO 4.5 for windows (Wageningen, the Netherlands).

RESULTS

1- Thiacloprid degradation

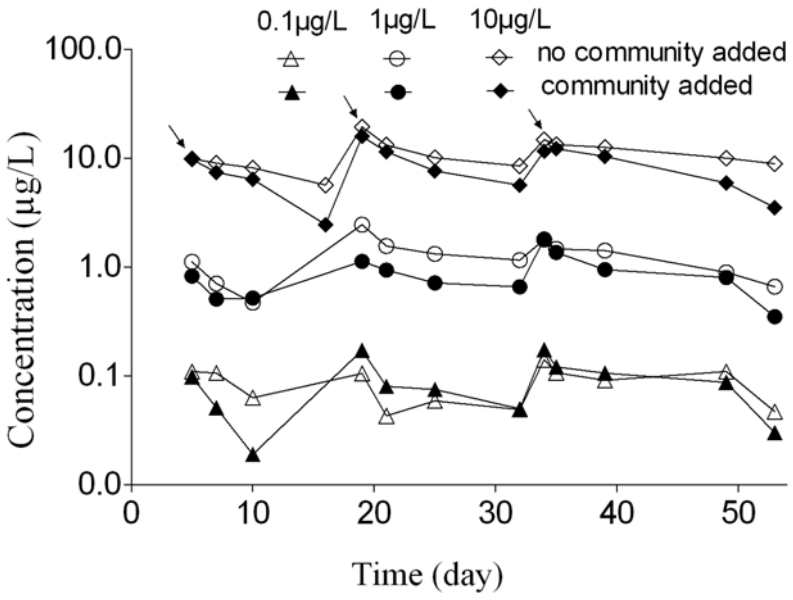


Fig. 1 Thiacloprid degradation in "no community added" and "community added" microcosms. ↘ indicates from the left to the right, the first, second, and third exposures of ponds to thiacloprid insecticide.

Measured and nominal concentrations were within the same range (Fig. 1). For example two hours after the first exposure, the measured concentrations were 0.11, 1.12, and 9.89µg/L in "no added community" setup, and 0.1, 0.82, and 9.76 µg/L in "added community" setup, corresponding to the nominal concentrations 0.1, 1 , and 10µg/L, respectively (Fig. 1). Peak concentrations of thiacloprid were detected in the water column of microcosms after each application (Fig 1). Following 20 days after the last exposure, thiacloprid was still detected in the water column of all treatments, including microcosms with the lowest initial concentration levels of 0.14 and 0.17 in the setups "no community added" and "community added", respectively (Fig. 1).

2- Effect of the community on oviposition and larval abundance of *Cx. pipiens*

In general, the oviposition was higher in "no community added" than in "community added" setups (Table 1). For example, the difference observed in oviposition between controls of "no community added" and "community added" microcosms was significantly different (Table 1; t-test, $P < 0.0001$). Similarly to oviposition, larvae of *Cx. pipiens* were more abundant in "no community added" than in "community added" microcosms (Fig. 2). For example, the difference observed in larval abundance between controls of "no community added" and "community added" microcosms was significantly different (Fig. 2; t-test, $P < 0.0001$). This result showed that the community added had a strong negative influence on oviposition and larval abundance of *Cx. pipiens*.

Table 1 Abundances (median of replicates) of eggs for all sampling days in "no community added" (n = 16) and in "community added" (n = 16) ponds.

Treatment	Community added	Time (day)													
		4	7	9	12	18	20	22	25	28	32	34	36	40	43
Control	no	6	3	7.5	10	3	3	2.5	0.5	1	2	0.5	0.5	1	1
	yes	0	0	0	0.5	0	0	0	0	0	0	0.5	0	0	0
0.1 µg/L	no	6.8	3.3	5.5	6.8	2.5	2	1	1	0	3	0	1	1	2
	yes	0	0	0	0.5	0	0	0.5	0	0	0	0	0	0	0
1 µg/L	no	7.5	3	4.5	6.8	1.8	1.5	0.5	0	2	5	2	1.5	3.5	1
	yes	0.3	0	0	1.8	0.3	0	1	0	0	0	0	0	0	0
10 µg/L	no	6.3	5	9	13	3.3	1.5	2	2	6	9.5	7.5	4	7.5	4.5
	yes	0	0	0	1.3	0	0	0	0	1	2	0	0.5	0	0.5

3- Ecological effects of thiacloprid

3.1- Effects of thiacloprid on the abundances of larval *Cx. pipiens* and their associated invertebrate community

Exposure to thiacloprid showed no effect on the abundance of *Cx. pipiens* at concentrations lower than 10 µg/L (Fig. 2). At 10 µg/L, the larval abundance of *Cx. pipiens* showed contrary effects in the presence and in the absence of added community (Fig. 2). In the setup "no added community", the larval abundance of *Cx. pipiens* decreased significantly after the second and the third exposures to 10 µg/L (Dunnett's test: $P = 0.023$). In the setup "community added" and in comparison to the control, the larval abundance of *Cx. pipiens* increased significantly in the 10-µg/L treatment on days 32 and 34 (i.e. 13 days following the second exposure; Fig. 2, Dunnett's test: $P < 0.0001$).

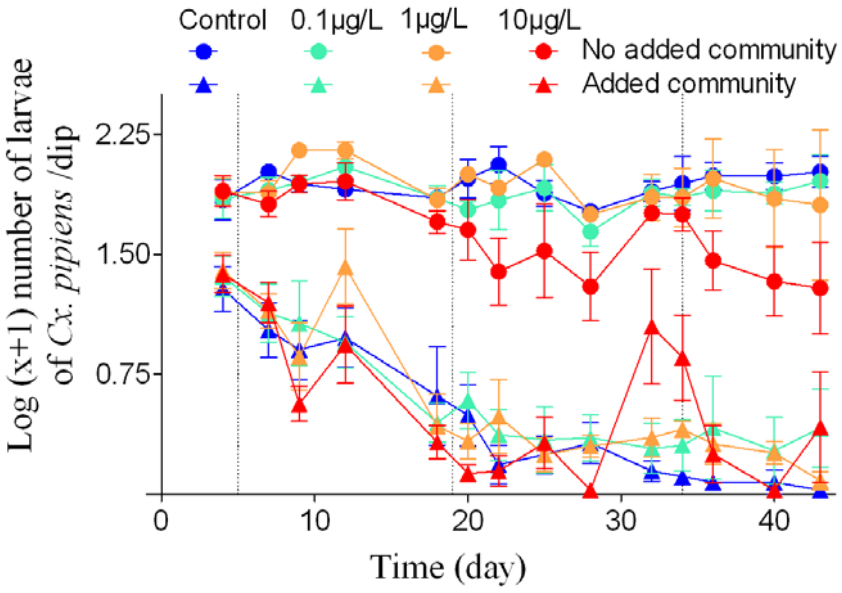


Fig. 2 Abundance variations of larvae of *Cx. pipiens* in "no community added" and "community added" microcosms. The vertical dashed lines from the left to the right indicate the first, second, and third exposures of ponds to thiacloprid insecticide.

Furthermore, we performed a principal response curve (PRC) on datasets of the "community added" setup to investigate the influence of thiacloprid on the structure of the community including larvae of *Cx. pipiens* and their associated invertebrate taxa. Owing to the differences in the sampling frequency, we used the moving average for *Cx. pipiens* with the means of records of larval abundances preceding each sampling day of their associated taxa.The results showed no significant effects at concentration lower than10 µg/L. By contrast in the concentration of 10 µg/L of thiacloprid, large deviations from the control were detected after the second exposure (Fig. 3, Monte Carlo permutations tests for each sampling day, Table 2).

Species weights (b_k) showed that Ostracoda (b_k = 2.2), Copepoda (b_k = 1.5), larvae of Chironomidae (b_k = 1.3), and *Hydra* sp. (b_k = 0.9) were the main taxa related

to the general pattern observed on the PRC (Fig. 3). Thus, those taxa were more affected than Acaria, Arcellinidae, Cladocera, Collembola, and larvae of *Cx. Pipiens* and Odonata (Fig. 3, $b_k < 0.5$).

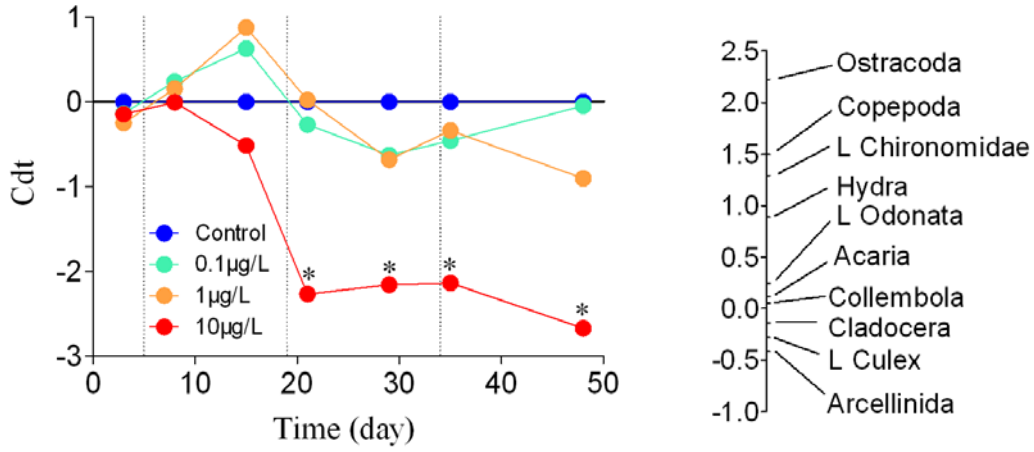


Fig. 3 Principal Response Curves (PRC) and species weights (on the right) showing the effects of thiacloprid on the structure of invertebrate communities in "community added" ponds. The y-axis presents the canonical coefficients (Cdt) and displays the differences between controls and the concentrations of thiacloprid of 0.1, 1, and 10µg/L. Species weights (b_k) express the link between each taxa and the PRC. The vertical dashed lines from the left to the right indicate the first, second, and third exposures of microcosms to thiacloprid insecticide. " * " stands for significant effects ($P < 0.05$) of thiacloprid following Monte Carlo Permutation tests (see details in Table 2).

Table 2 Results of the Monte Carlo permutation tests (P-values) performed per sampling day for invertebrate communities in "community added" ponds (n=16) during the study period.

Time	All	0.1 µg/L	1 µg/L	10 µg/L
Day 3	NS	NA	NA	NA
Day 8	NS	NA	NA	NA
Day 15	NS	NA	NA	NA
Day 21	0.0040 (22.3%)	NS	NS	0.0220 (42.7%)
Day 29	0.0020 (26.3%)	NS	NS	0.0220 (45.3%)
Day 35	0.0040 (22.0%)	NS	NS	0.0220 (43.6%)
Day 48	0.0020 (24.4%)	NS	NS	0.0220 (41.3%)

NS: not significant (i.e. P>0.05)
NA: not applicable
( ): Percentages of the variations explained by thiacloprid exposure

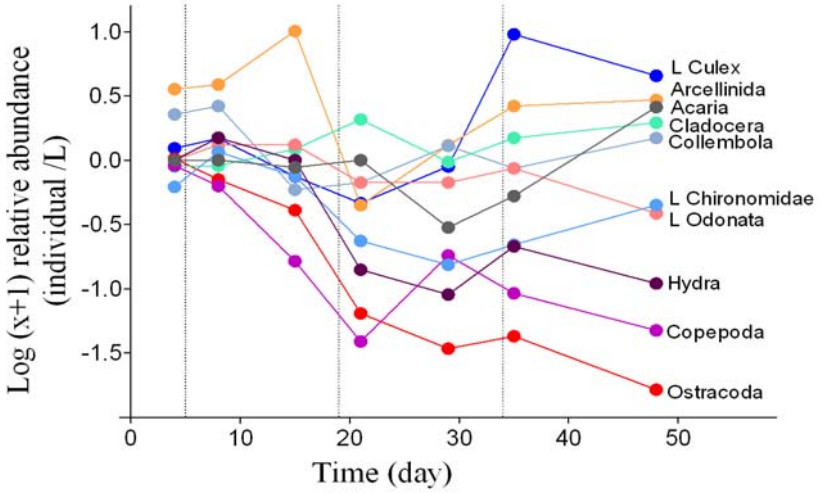


Fig. 4 Abundance variations in the setup "community added", given as a deviation of the abundance from the control, of larvae of *Cx. pipiens* and their associated invertebrate taxa in the concentration of thiacloprid of 10 µg/L. The vertical dashed lines from the left to the right indicate the first, second, and third exposures of microcosms to thiacloprid insecticide.

The development of taxa after exposure to thiacloprid at the concentration of 10 µg/L is shown in details in Fig 4. The development of taxa is given as a deviation of the abundance from the control. Indeed, all invertebrate taxa which were affected in the PRC (i.e Ostracoda, Copepoda, *Hydra* sp. and larvae of Chironomidae; Fig. 3) had their abundances, after the second exposure, lower in the concentration of thiacloprid of 10µg/L than in the control (Fig. 4).

3.2- Relationship between long term development of taxa in the setup "added community" and their individual sensitivity to thiacloprid

Fig. 5 shows the relationship between the long-term development of taxa after exposure to thiacloprid at the concentration of 10 µg/L and their individual LC50 values found in a laboratory experiment (unpublished data, Appendix 1). We considered in this analysis only *Cx. pipiens* and taxonomic groups which are well known to interact with mosquito larvae as competitors for food sources (e.g. Ostracoda, Cladocera, and larvae of Chironomidae, Copepoda) or as predators (e.g. Copepoda and larvae of Odonata). The long term development was calculated as the difference in the abundance between the pre treatment (on day 3) and the end of the experiment (on day 48).

Except for larvae of *Cx. pipiens* and Chironomidae, the long-term development of the other taxonomic groups (i.e. Ostracoda, Copepoda, Cladocera, and larvae of Odonata) in the concentration of thiacloprid of 10 µg/L was positively correlated with their LC50 (Fig. 5). Larval abundances of *Cx. pipiens* and Chironomidae were less affected by thiacloprid in the microcosm experiment in comparison to their highest LC50 at the individual level (Fig 5). This result suggests that the recovery potential,

after the stress generated by thiacloprid, was stronger for *Cx. pipiens* and Chironomidae than for the other sensitive taxa (e.g. Ostracoda, Copepoda).

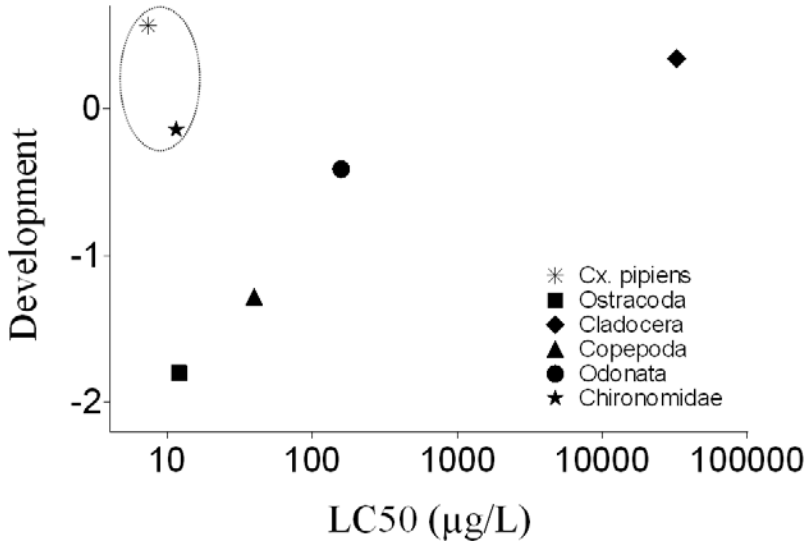


Fig. 5 Associations between the long term development in the 10-µg/L treatment of the setup "community added", and the median lethal concentration (LC50) of thiacloprid from a laboratory test for *Cx. pipiens* and their associated potential competitors (i.e. Ostracoda, Cladocera, larvae of Chironomidae, and Copepoda) and predators (Copepoda, and larvae of Odonata). The test organisms for the laboratory test were freshly collected from non contaminated microcosms of "community added" setup in the outdoor microcosm experiment. Except for larvae of *Cx. pipiens* and Chironomidae (encircled), the long term development of invertebrate taxa in the concentration of thiacloprid of 10 µg/L was positively associated with their LC50 during the individual test.

3.3- Relationships between increase in larval abundance of *Cx. pipiens*, the structure of the community, and thiacloprid treatment in the "community added" setup

In the 10 µg/L-treatment of the "community added" setup, a significant increase in the abundance of larvae of *Cx. pipiens* was observed on day 32 (Fig. 2). Studies have demonstrated that mosquitoes like in general breeding sites that contain few competitors for food sources and predators (Duquesne et al. 2010, Meyabeme et al. 2010). Hence, we carried out a principal component analysis (PCA) on dataset of the "community added" setup to identify relationships between *Cx. pipiens* (i.e. abundance larvae and oviposition), and their potential associated natural enemies (i.e. abundances of competitors and predators). We considered all treatments levels (control, 0.1, 1, and 10 µg/L) about day 32 for oviposition and larvae of *Cx. pipiens*, and about the previous sampling day (day 29) for their associated natural enemies. Thiacloprid treatment was passively projected on the ordination plot as explainable variable (Fig. 6).

The results showed that there was a positive relationship between oviposition and the abundance of larvae of *Cx pipiens* (Fig 6). The oviposition and the abundance of larvae of *Cx. pipiens* were associated negatively to the abundances of Copepoda, Ostracoda, larvae of Chironomidae and Odonata (hereafter antagonists) on one hand, and positively to thiacloprid concentrations on the other hand (Fig. 6). There was a negative relationship between antagonists and thiacloprid concentrations (Fig. 6).

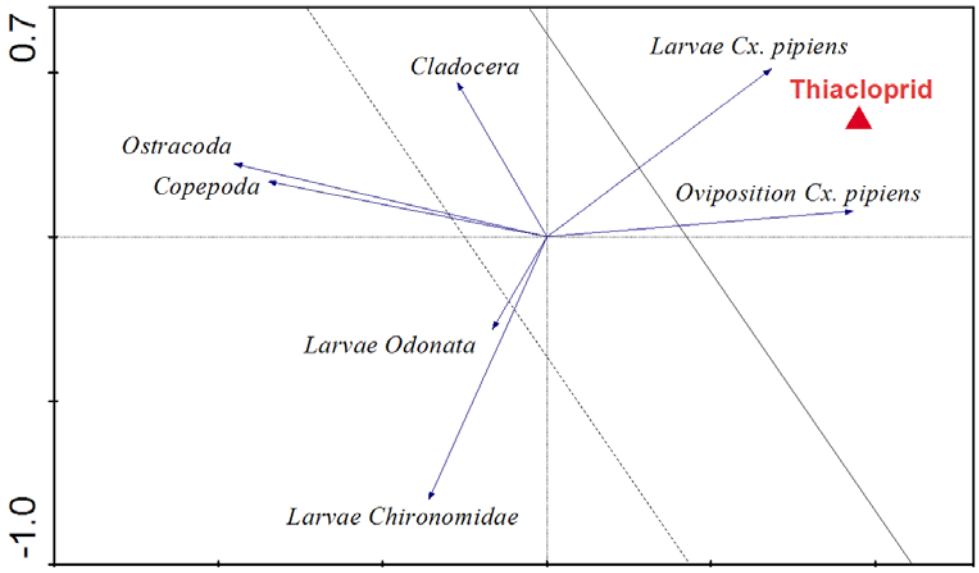


Fig. 6 Ordination plot for principal component analysis (PCA) performed on data about the increase in the abundance of larvae of *Cx pipiens* on day 32 and the abundances of their potential competitors and predators on the previous sampling day (i.e. day 29). Data for all treatments (i.e. control, 0.1, 1, and 10 µg/L) were considered in this analysis. Arrows on the right side of the solid line show the positive correlation between thiacloprid, abundance of larvae and oviposition of *Cx. pipiens*. Arrows on the left side of the dashed line show taxa that were associated negatively with the abundance of larvae of *Cx. pipiens*. Arrows between dashed and solid lines show no correlations with *Cx. pipiens*.

Using linear regressions, we tested the significance of the relationships firstly between the abundances of larvae of *Cx. pipiens* and the pooled data of antagonists and secondarily, between the abundance of pooled data of antagonists and thiacloprid concentrations. We found that 32% of the variation in the increase of the abundance of larvae of *Cx. pipiens* was explained significantly by the decrease in the abundance of

pooled antagonists (Fig. 7A, P = 0.023); and that thiacloprid treatment could explain significantly 78% of the variation observed in the abundance of antagonists (Fig. 7B, P < 0.0001). This result suggests that the increase in the abundance of larvae of *Cx. pipiens* observed on day 32 in the concentration of thiacloprid of 10 µg/L was obviously a consequence of the decrease in the abundances of antagonists by thiacloprid.

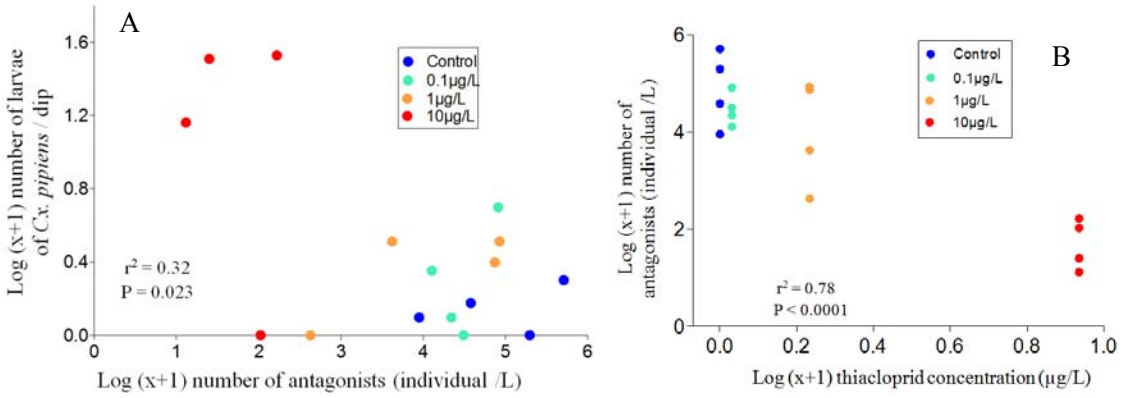


Fig. 7 Relationships between the abundances of larvae of *Cx. pipiens* (day 32) and their antagonists (day 29) (A), and the abundance of antagonists (day 29) and thiacloprid concentrations (day 25) (B), "community added" setup.

3.4- Abiotic parameters and chlorophyll

Dissolved oxygen, pH, electric conductivity, and total dissolved solids did not show any treatment related effect during the course of the experiment in "no community added" and "community added" setups. The range of variations of dissolved oxygen, pH, electric conductivity, total dissolved solids, and chlorophyll are given in Table 3.

Table 3 Mean values and range of variations of dissolved oxygen (DO), pH, electric conductivity (EC), total dissolved solids (TDS), and Chlorophyll (Chl) for the different treatment levels in "no community added" (n =16) and "community added" (n =16) setups.

Factor		Control		0.1 µg/L		1 µg/L		10 µg/L	
		mean	min - max	mean	min - max	mean	min - max	mean	min - max
No community added	DO (%)	76	32 -130	81	51-127	81	43-151	77	46-138
	pH	8.19	6.97- 9.61	8.17	7.04 - 8.95	8.15	7.01- 9.69	8.06	7.06- 9.18
	EC (µS/cm^2)	548	462 -707	531	427-648	537	455 - 678	550	392 - 679
	TDS (mg/L)	367	273 - 469	353	268 - 431	359	257- 447	368	281- 456
	Chl (RFU)	5.9	0.9 - 20.3	4	0.7 – 10	4.8	0.5 – 21.2	3.6	0.8 – 17.6
Community added	DO (%)	95	50 -154	106	62 - 166	100	58 - 154	120	62 - 187
	pH	8.17	6.61- 9.56	8.22	6.49-9.34	8.29	6.63 -9.30	8.71	6.85 - 9.77
	EC (µS/cm^2)	457	336 -556	450	351-556	464	292 - 621	443	286 -545
	TDS (mg/L)	306	220 - 400	301	217-392	312	216 - 417	294	195 - 385
	Chl (RFU)	0.6	0.3 – 1.9	0.8	0.4 – 5.5	0.8	0.2 – 6.5	0.6	0.3 – 1.7

Concerning turbidity, there was a significant difference in the turbidity content between control and the concentration of thiacloprid of 10 µg/L after the second exposure in the setup "no community added" (Fig 8A, Dunnett's test: P = 0.008), and at the end of the experiment on day 48 in the setup "added community" (Fig 8B, Dunnett's test: P = 0.043). No significant difference was observed between control and the concentrations of thiacloprid of 0.1 and 1µg/L in the "no community added", as well as in the "community added" setups.

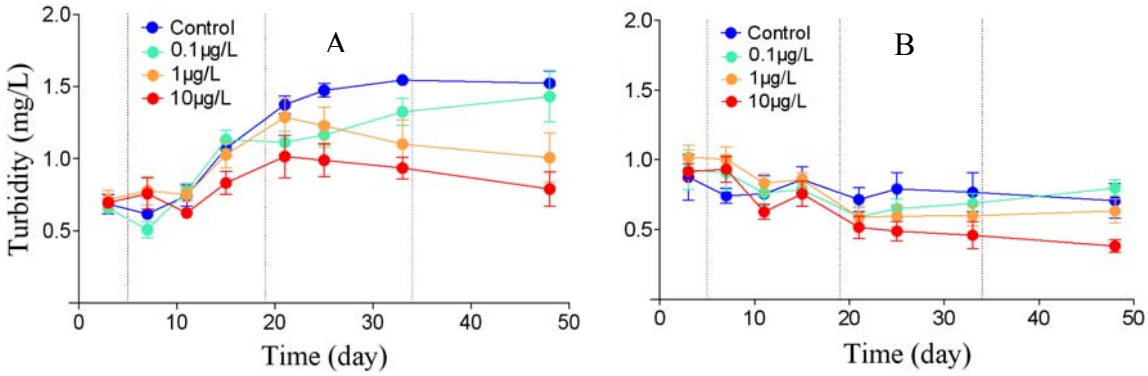


Fig. 8 Variations in the content of turbidity in "no community added" (A) and "community added" (B) setups. The vertical dashed lines from the left to the right indicate the first, second, and third exposures of microcosms to the insecticide thiacloprid.

Turbidity is caused by the presence in the water column of suspended matters, whether mineral (e.g. soil particles) or organic (e.g. algae, other microscopic organisms) (Michaud 1991; EPA 1999). Therefore, the decrease of turbidity in the concentration of 10µg/L is likely a consequence of the reduction of the abundances of *Cx. pipiens* and associated organisms by thiacloprid.

DISCUSSION

Degradation of thiacloprid

Around 20 days following the last exposure, thiacloprid was still detected from the water column of all treated microcosms, including microcosms with initial concentration < 0.2 µg/L. However, Beketov et al. (2008) noted a faster degradation of this insecticide in a stream mesocosm system. These authors found no detectable amounts of thiacloprid (< 0.01 µg/L) in the water column after nine days following the application of initial concentration 2.83 µg/L. The degradation velocity of thiacloprid might differ between lentic and lotic ecosystems. Owing to the paucity of studies on the behaviour of thiacloprid in surface water, the processes behind these variations were not clear to us.

Differential occurrence of oviposition and larvae of *Cx. pipiens* in "no community added" and in "community added" setups

The high density of invertebrate communities was a strong influential factor for the development of *Cx. pipiens* over time. Invertebrate taxa in our microcosms comprised potential competitors for food (e.g. Cladocera, Ostracoda, larvae of Chironomidae) and predators (e.g. the Copepoda Cyclopoida, larvae of Odonata). Several authors have established the negative influence of competitors and predators on the oviposition behaviour and larval abundances of mosquitoes (Kumar and Hwang 2006; Marten and Reid 2007; Duquesne et al. 2010; Vonesh and Blaustein. 2011). For example, the oviposition deterring effect by predators (e.g. larvae of Odonata) on gravid female mosquitoes (e.g. *Culiseta longiareolata*) was shown by Stav et al.

(1999) and Stav et al. (2000). Duquesne et al. (2010) found in a two-species microcosm experiment that the oviposition and the abundance of larvae of *Cx. pipiens* were low in conditions of high abundance of *Daphnia magna*. Marten and Reid (2007) reported the adverse impacts of Cyclopoida species on the abundance of mosquito larvae (e.g. species of *Aedes*, *Anopheles*, and *Culex*). Thus, our result about higher occurrence of larvae and oviposition of *Cx. pipiens* in "no community added" than in "community added" microcosms was obviously a consequence of the higher competing and predatory influence in the latter setup.

Effects of thiacloprid on the abundances of larval *Cx. pipiens* and their associated invertebrate community

"No community added" setup. The direct effects of thiacloprid on larval abundance of *Cx. pipiens* could be seen in this setup. Based on the individual LC50 (14 day) of thiacloprid of 6.04 µg/L for larvae of *Cx. pipiens* after a short term exposure (24-h) (Beketov and Liess 2008), we would expect a strong lethal effect on the abundance of larvae of this mosquito in microcosms treated with thiacloprid concentration of 10 µg/L. Instead, we observed no visible effect on the abundance of larval *Cx. pipiens* related to the first exposure to thiacloprid, and only a partial decrease after the second and third exposures. In our outdoor study, ponds were exposed to natural conditions and larval populations of *Cx. pipiens* were subjected potentially to be regenerated through new recolonisations by terrestrial adults. This flux might not allow the observation of stronger effects of thiacloprid on larval abundance of *Cx. pipiens* in the setup "no community added". The present result suggests that the perception of the impact of a pesticide at individual level can be shifted under natural conditions.

Therefore, the use of individual tests is not sufficient to predict the effects of pesticides for larval control of mosquitoes, other approaches such as preliminary tests in the outdoor in areas with high occurrence of mosquitoes may be more reliable and realistic.

"Community added" setup. We found that larval abundance and oviposition of *Cx. pipiens* were correlated positively with thiacloprid concentrations and negatively to their antagonists (i.e Ostracoda, Copepoda, and larvae of Chironomidae and Odonata) and that, the antagonists of *Cx. pipiens* were correlated negatively with thiacloprid treatments. The decrease in the abundances of Ostracoda, Copepoda, and larvae of Chironomidae and Odonata led as well to the release of predation and interspecific competition pressures on larval *Cx. pipiens*. Thus, the role of antagonists in regulating mosquito populations discussed above was weakened and less effective in the concentration of thiacloprid of 10µg/L; as a result, there was a long term increase in the abundance of larvae of *Cx. pipiens* in this concentration. Pingali and Roger (1995) reported similarly a resurgence of larvae of *Cx. tarsalis* due to reduced predation by the fungicide triphenyltin hydroxide and the chitin synthesis inhibitor benzoylphenyl urea in rice fields. Our outcome supports the idea that, when the spectrum of a pesticide is enlarged to non targets, it can create suitable conditions for the development of opportunistic pests such as mosquitoes due to the losses of predators and competitors.

Recovery potential of invertebrate taxa and influence of life traits

Ostracoda, Copepoda, and Larvae of *Cx. pipiens* and Chironomidae showed a high sensitivity to thiacloprid at individual level in comparison to Cladocera and larvae

of Odonata (unpublished data). Among the sensitive taxa, only larval populations of *Cx. pipiens* and Chironomidae exhibited a good recovery potential in the outdoor microcosm experiment. In comparison to the life cycle of Ostracoda and Copepoda which is exclusively aquatic (Ward and Whipple 1959), the immature stages (i.e. eggs, larvae, and pupae) of *Cx. pipiens* and Chironomidae occur in aquatic ecosystems and adults are terrestrial (Armitage et al. 1995, Becker et al. 2003). Studies have demonstrated that an adult mosquito (Becker et al. 2003) or Chironomidae (Armitage et al. 1995) can lay several hundred eggs at once on the water surface of a breeding site. As a consequence, the recolonisation of microcosms by terrestrial adults during the present experiment, might explain the better recovery of larval populations of *Cx. pipiens* and Chironomidae in comparison to Ostracoda and Copepoda.

In river systems, the recovery of sensitive taxa after a toxicant stress occurs from uncontaminated upstreams (Schäfer et al. 2007) among others; in this case, both strict- and semi- aquatic taxa have good chances of recolonisation. However in close systems such as ours, the most important trait for the recovery seems to be the presence of adults outside the water. The relevance of a trait-based approach, for distinguishing toxicant effects at the community level, was previously demonstrated for river systems (Liess and Von der Ohe 2005, Beketov et al. 2009). The results of the present study show an example of an important trait that can be valuable for risk assessment studies about close systems (e.g. temporary water bodies).

CONCLUSION

Overall, our study showed that the response of larval populations of *Cx. pipiens* to the insecticide thiacloprid differed according to the ambient conditions. In this example, thiacloprid affected negatively the abundance of larval *Cx. pipiens* in the absence of interspecific interactions in "no community added" microcosms. But in "community added" microcosms, the long term development of larval *Cx. pipiens* was influenced positively by thiacloprid as a consequence of the non target effects. A good mosquito larvicide should be efficient, fast-acting, and safe for non target species, especially for competitors and predators that can regulate naturally the abundance of mosquito larvae. We recommend multispecies toxicity tests including mosquito larvae and their natural enemies as a tool to improve the risk assessment of mosquito larvicides. We also showed that the presence of adults outside the water appears to be an important trait for sensitive taxa to recover from thiacloprid effects in the microcosms. This result is very useful for a trait-based approach in risk assessment for predicting toxicant effects at the community level in lentic ecosystems.

ACKNOWLEDGEMENTS

We are grateful to Mrs Ingrid Ränker for helping in the measurements of abiotic parameters and Mr Klaus Seyfarth for his active participation in the establishment of microcosms. This work was made possible with the financial support

of the German Academic exchange Service (DAAD) and the Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany.

REFERENCES

Armitage PD, Cranston PS, Pinder LCV (1995) The Chironomidae: the biology and ecology of non-biting midges. Chapman and Hall, London, Great Britain

Barnard R (2003) West nile disease and its control. Pesticide Outlook 14 (2):76-79

Becker N, Petrić D, Zgomba M, Boase C, Dahl C, Lane J, Kaiser A (2003) Mosquitoes and their control. Kluwert Academic/Plenum Publishers, New York, N.Y., USA

Beketov MA, Foit K, Schäfer RB, Schriever CA, Sacchi A, Capri E, Biggs J, Wells C, Liess M (2009) SPEAR indicates pesticides effects in streams-comparative use of species-and family-level biomonitoring data. Environ Pollut 157:1841-1848

Beketov MA, Liess M (2008) Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. Environ Toxicol Chem 27 (2):461-470

Beketov MA, Schäfer RB, Marwitz A, Paschke A, Liess M (2008) Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: effect concentrations and recovery dynamics. Sci Total Environ 405:96-108

Chase JM, Knight TM (2003) Drought induced mosquito outbreaks in wetlands. Ecol Lett 6:1017-1024

Duquesne S, Kroeger I, Kutyniok M, Liess M (2010) The potential of Cladocera as controphic competitors of the mosquito *Culex pipiens*. J. Med. Entomol. (in press)

Durand JR, Lévêque C (1980). Aquatic flora and fauna of sahelo-Sudanese Africa, Volume I, Office for Overseas Scientific and Technical Research (O.R.S.T.O.M), Paris, France

EPA [Environmental Protection Agency] (1999). Importance of turbidity. EPA Guidance Manual Turbidity Provisions. http://www.epa.gov/safewater/mdbp/pdf/turbidity/chap_07.pdf. Accessed 21 December 2009

Hemingway J, Beaty BJ, Rowland M, Scott TW, Sharp BL (2006) The innovative vector control consortium: improved control of mosquito-borne diseases. Parasitol today 22 (7):308-312

Kumar R, Hwang JS (2006). Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. Zool stud 45(4):447-466.

Leps J, Smilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, UK

Liess M, Von Der Ohe PC (2005) Analysing effects of pesticides on invertebrate communities in streams. Environ Toxicol Chem 24 (4):954-965

Marten GG, Reid JW (2007) Cyclopoid copepods. J Am Mosq Control Assoc 23:65-92.

Mendis K, Rietveld A Warsame M, Bosman A, Greenwood B Wernsdorfer WH (2009) From malaria control to eradication: the WHO perspective. Trop Med Int Health 14 (7):802-809

Meyabeme Elono AL, Liess M, Duquesne S (2010) Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetlands areas in Germany (in press)

Michaud JP (1991) A citizens' guide to understanding and monitoring lakes and streams. Publ. #94-149. Washington State Department of Ecology, Publications Office, Olympia, WA, USA 360-407-7472

Pingali LP, Roger P (1995) Impact of pesticides on farmer health and the rice ecosystem. Kluwer Academic Publishers, Boston, Massachusetts, USA

Ramirez JL, Garver LS, Dimopoulos G (2009) Challenges and Approaches for mosquito targeted malaria control. Curr Mol Med 9:116-130

Reiter P (2010) Yellow fever and dengue: a threat to Europe? Euro. Surveill. 15 (10):pii=19509. http://www.eurosurveillance.org/ViewArticle.aspx?Articled=19509. Accessed 19 May 2010

Schäfer RB, Caquet T, Siimes K, Mueller R, Lagadic L, Liess M. (2007) Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Sci Total Environ 382:272-285

Schmuck R (2001) Ecotoxicological profile of the insecticide thiacloprid. Pflanzenschutz-Nachr Bayer 54:161-184

Schwab H (1995). Freshwater animal. 1st ed. Klett Schubuch, Stuttgart, Germany.

SPSS Inc. (2009) PASW Statistics 17.0.3 for Window. SPSS Inc., Chicago, Illinois.

Stav G, Blaustein L, Margalithy J (2000) Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta longiareolata*

(Diptera: Culicidae) and community structure in temporary pools. J Vector Ecol 25:190-202

Stav G, Blaustein L, Margalit J (1999) Experimental evidence for predation risk sensitive oviposition by a mosquito, *Culiseta longiareolata*. Ecol Entomol 24:202-207

Streble H, Krauter D (2006) The life in water, Microflora und Microfauna of freshwaters. 10th ed. Franckh-Kosmos, Stuttgart, Germany

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P (2003) Freshwater invetebrates, systematic, biology, ecology. CNRS, Paris, France.

Tomizawa M, Talley TT, Maltby D, Durkin KA, Medzihradszky KF, Burlingame AL, Taylor P, Casida JE (2007) Mapping the elusive neonicotinoid binding site. Proc Natl Acad Sci USA. 104 (21):9075-9080

USEPA [U.S. Environmental Protection Agency] (2003) Office of Prevention, Pesticides and toxic Substances. Fact sheet for thiacloprid. http://www.epa.gov/opprd001/factsheets/thiacloprid.pdf. Accessed 02 June 2007

Van den Brink PJ, ter Braak CJF (1999) Principal response curves: Analysis of time-dependent multivariate responses of a biological community to stress. Environ Toxicol Chem 18:138-148

Vonesh, J.R, & L. Blaustein. 2010. Mosquito oviposition site selection in response to predation risk: Implications for vector control. Israel Journal of Ecology and Evolution (in press).

Ward HB, Whipple GC (1959) Fresh-water biology, 2nd ed. John Wiley & Sons, Inc. New York & London, USA.

WHO [World Health Organization] (2005) World malaria report 2005. World Health Organization and UNICEF. Geneva, Switzerland.

WHO [World Health Organization] (1975) Manual on practical entomology in Malaria. Part II: methods and techniques. World Health Organization Offset Publication No. 13. Geneva, Switzerland: WHO.

Appendix 1 Median lethal concentrations (LC50) of thiacloprid derived from a laboratory test performed on larvae of *Cx. pipiens* and their potential competitors (i.e. Ostracoda, Cladocera, larvae of Chironomidae, and Copepoda) and predators (Copepoda and larvae of Odonata). The test organisms for the laboratory test were taken from non contaminated ponds in the "community added" setup one day before the beginning of the test itself.

Taxa	LC50 (µg/L)	Duration (day)	Reference
L Culex	7,38	6	Meyabeme et al. Unpublished data
L Chironomidae	11,55	4	Meyabeme et al. Unpublished data
Ostracoda	12,12	18	Meyabeme et al. Unpublished data
Cyclopoida	39,81	6	Meyabeme et al. Unpublished data
L Odonata	158,44	6	Meyabeme et al. Unpublished data
Cladocera	32608,24	6	Meyabeme et al. Unpublished data

Chapter IV

Invertebrate density sustaining the efficiency of Bti based VectoBac in mosquito control, a case study in Cameroon

Alvine Larissa MEYABEME ELONO[1], Matthias LIESS[1*], and Sabine DUQUESNE[1, 2]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of System Ecotoxicology, Permoserstrasse 15, D-04318 Leipzig, Germany

[2]UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, D-04318 Leipzig, Germany

American Journal of Tropical Medicine and Hygiene, in preparation for submission.

Abstract We investigated the influence of invertebrate communities for supporting the control of mosquito larvae with the biological insecticide VectoBac based on *Bacillus thuringiensis* var *israelensis* (Bti) (4 g/m^3). The study took place in temporary ponds in Cameroon (Central Africa) and included (i) a "Bti and community" experiment (duration, two weeks) with three setups, i.e., only added community ("com"), only VectoBac ("Bti"), and both added community and VectoBac ("comBti"); and (ii) a "community density" experiment (duration, three weeks) including three setups, all treated with VectoBac but with added community at different density (i.e. "low", "medium", and "high"). The two mosquito mosquito taxonomic groups identified were *Anopheles* spp. and *Culex* spp. After 24 h following the application of VectoBac, larval abundances of these mosquitoes were reduced from about 78 to 99% in Bti-treated ponds of both experiments. The recovery of mosquito populations in these ponds was fast and substantial in low community density conditions ("Bti" and "low"), moderate in medium community density ("medium) and, nonexistent in high community density ("comBti" and "high"). In the setup treated with added community alone ("com"), the abundance of mosquito larvae first increased initially and then decreased progressively till the end of the experiment. These results suggest that the treatment with VectoBac alone was efficient for a shorter time whereas added community alone was efficient in a longer term. However, the combination of VectoBac and added community was efficient in shorter and longer terms. This implies that the efficacy of larval control of mosquitoes by Vectobac was improved with high community density.

Key words: *Anopheles* spp., *Culex* spp., Competitors and predators, temporary ponds, Cameroon

INTRODUCTION

Since the discovery of mosquitoes as vectors of diseases (Ross, 1897), several control strategies have been developed and implemented (Ramirez et al 2009). Before the years 1960s, chemical insecticides were largely used in mosquito control. However, the resistance by the target populations to these insecticides (Hemingway & Ranson 2000) and the appearance of unwanted damages to the environment (Becker et al. 2003) led to the exploration of alternative methods such as biological control. Biological control implies the use of beneficial organisms (e.g. bacteria, parasites, predators, competitors) for reducing pests or mitigating pest effects.

Larvicides based on the bacterium *Bacillus thuringiensis* var. *israelensis* (Bti) are considered to be among the safest pesticides in pest control programs of the time (Boisvert 2005). Bti has a great success in mosquito control in many countries worldwide (Becker et al. 2003, Lacey 2007). Mosquito larvae are affected and killed 24 hours following ingestion of Bti spores (Amalraj et al. 2000, Aldemir 2007, Kahindi et al. 2008). Nevertheless, Bti become quickly unavailable to filter feeding mosquito larvae due to the fast adsorption of spores to the sediments. As a consequent, repeated applications are necessary to prevent the resurgence of the next generations of mosquitoes in treated biotas (Hougard & Back 1992, Becker et al. 2003). Besides, the susceptibility of non target organisms to Bti (e.g. Chironomidae, Lepidoptera, *Chlorella* sp., *Closterium* sp.) has been reported (Boisvert & Boisvert 2000). However, natural enemies of mosquito larvae such as Cladocera, Cyclopoida, Notonectidae and Dytiscidae (Knight et al. 2004, Kumar and Hwang 2006, Marten and Reid 2007) are

not affected by Bti at recommended dosages for operational treatments (Boisvert & Boisvert 2000).

In African countries, many mosquitoes (e.g. *Anopheles*, *Culex*) are involved in the transmission of dangerous diseases like malaria and lymphatic filariasis (Fontenille & Carnevale 2006, Camargo 2008). During the first rainfalls in tropical regions, temporary waters are formed in the ground depressions and are used as breeding sites by mosquitoes (e.g. *Anopheles* species) (Chaves & Koeenradt 2010). This might explain the strong positive correlation that is observed between rainfall and abundances of mosquitoes in countries such as Cameroon (Barbazan et al. 1997, Bigoga et al. 2007, Atangana et al. 2009), Ghana (Tuno et al. 2010), Nigeria (Oyewole et al. 2007), and Senegal (Faye et al. 1997).

Moreover, human activities (e.g. sand pits, agricultural irrigation) contribute for amplifying environmental conditions of the breeding of mosquitoes (Mutuku et al. 2006, Simard et al. 2009). The aim of the present study is to assess the influence of aquatic invertebrate communities, which include competing and predatory taxa, in sustaining a Bti-based treatment for the larval control of mosquitoes in human-made breeding sites. We conducted (i) a "Bti and community" experiment to compare the efficacy of the treatments by Bti alone, community alone, and both Bti and community in combination; and (ii) a "community density" experiment to assess the influence of the density of the community in the re-establishment of mosquito larvae in ponds after Bti treatment.

The study took place during the small rainy season (March-June 2008) in the wetland of the stream Mbomo that waters the periurban area of Mfou, Cameroon (Central Africa). One activity of the neighbouring residents of that wetland is the

domestic agriculture of green vegetables. Some of the small ponds that are created for watering vegetables were used in the present study. The hypothesis was that the abundance of mosquito larvae after Bti application will be much lower in treatments combining Bti and high community density than treatments with either Bti alone or high community density alone. The results derived from the present study shall be useful for control strategies of mosquitoes based on Bti in peridomestic temporary ponds during the rainy season.

MATERIALS AND METHODS

Study site

The investigation was conducted in Cameroon (Central Africa) during the small rainy season (April to June 2008). Study sites were located in the wetland of the stream Mbomo which waters Mfou (3°58'N 11°56'E), a peri-urban area close to the capital Yaoundé. The climate is characterized by two rainy seasons (March – June and August – November). The neighbouring residents use the wetland of the stream Mbomo for irrigation of green vegetables among other activities (e.g., digging of sand). At the end of the rainy season when the level of water drops down, small semi-natural ponds are created for irrigating plants. These ponds generally dry out in the course of the dry season. But following the first rainfalls of the next rainy season, they refill and contribute to the high occurrence in mosquito populations in the location Mfou.

Experimental design

We selected 16 ponds that are use by neighbouring residents for watering their vegetables. The first water and the soft layer of the sediments were emptied from all selected ponds so that initial conditions, in terms of quantity and quality of the community, are the same at the start of the experiment. Ponds were filled up again with water from rain and infiltration and left to be colonised by local mosquitoes.

Then, ponds were subjected to the treatments with *Bacillus thuringiensis* var *israelensis* (Bti) and/or community. The formulation of Bti which was used during this investigation was VectoBac. It was obtained from the German Mosquito Control Association –KABS (Waldsee, Germany). The rate of 4 g/m^3 of VectoBac was applied on the surface of ponds using an 8-L graduated and transparent air pressure sprayer SM-8A (Zhejiang, China). The local invertebrate communities from the surrounding ponds were used for setting the community condition in experimental ponds. This was realised by filtration of 100 L of water to 1 L using a 55-µm mesh. This operation was repeated several times and the 1-litre subsamples were pooled together into a single sample. This invertebrate concentrate was distributed in ponds in respect to the different community treatments. The taxonomic groups collected encompassed Ciliata, Rotifera, Ostracoda, Cladocera (i.e. *Ceriodaphnia* spp. and *Chydorus* spp.), Hydracarina, Collembola, Cyclopoida, *Hydra* sp. and insects larvae (i.e. Chironomidae, Ephemeroptera, Hydrophilidae, and Odonata). The identification keys which were used were those of Ward and Whipple (1966), Durand and Lévêque (1980), Schwab (1995), Becker *et al*. (2003), and Tachet *et al*. (2003).

“Bti and community” experiment

This experiment included three different setups, i.e. the biological pesticide VectoBac alone (“Bti”, n = 5), added community alone (“com”, n = 5), and added community + VectoBac (“comBti”, n = 5). Each pond of the setups “com” and “comBti”, received three times 500 mL of the invertebrate concentrate (obtained as described above) per m^3 of water. No community aliquot was added in “Bti” ponds. The treatments with VectoBac and added community were applied once after four days following the initiation of the experimental ponds. The treatment with added community was applied three hours before the treatment with VectoBac. Just before the VectoBac treatment, the mean numbers of invertebrate taxa in the setups “Bti”, “com”, and “comBti” were 79 ± 20, 167 ± 15, and 167 ± 21 individuals /L, respectively. This experiment lasted around two weeks from the initiation of ponds to the end of the monitoring.

“Community density” experiment

This experiment included three setups with VectoBac treatment and different community densities, i.e. “low” (n = 8), “medium” (n = 4), and “high” (n = 4) density. The aliquots of 500 mL of the invertebrate concentrate were introduced once, twice, and fourth per m^3 of pond water in the setups “low”, “medium”, and “high” community density, respectively. The treatment with added community was applied four days before the treatment with VectoBac to allow the establishment of the invertebrate populations. On the day of VectoBac treatment, the mean numbers of invertebrate organisms for the setups “low”, “medium”, and “high” community density were 72 ± 18, 88 ± 20, 170 ± 30 individuals/L, respectively. The “community density”

experiment lasted around three weeks from the initiation of ponds to the end of the monitoring.

Monitoring of mosquito larvae and associated invertebrates communities

In the two experiments, the abundances of mosquito larvae were recorded once before VectoBac treatment and at least two times per week afterwards. Mosquito larvae were sampled by dipping four times a volume of 250 mL at the edge of each pond (adapted standard technique, WHO 1975). The four samples were averaged and the abundance was expressed in number of larvae per dip. Counted larvae were immediately returned to the pond.

The abundances of invertebrate taxa associated with mosquito larvae were recorded once before VectoBac treatment and at least three times in the post treatment periods. Specifically, we took 10 samples of 250 mL from different sides and depths of each pond. The subsamples were pooled together into a single sample and gently stirred. One litre of this pooled sample was filtered through a 55-µm mesh and conserved in a 15-mL brown flask with a mixture ethanol:distilled water (70:30). The remaining part of the pooled sample was returned back to the original pond.

Physicochemical parameters

The physicochemical parameters were measured once before VectoBac treatment and twice in the post exposure period in the two experiments. Water temperature, pH, and total dissolved solids (TDS) were measured with an electronic multimeter ExStik EC500 (Walthman, USA). Dissolved oxygen (DO) was measured with an electronic oxymeter ExStik DO600 (Walthman, USA). The variables required

for calculating the water surface area and the volume (i.e. diameter and depth) were measured using a graduated ruler. For the measurements of Chlorophyll *a*, samples of 250 mL were filtered through a 0.45 µm mesh-size Whatmann GF/C glass fiber filter. This filter was kept in acetone during 24h in the dark and at 4°C for extraction. The optical density of the extract was recorded with a spectrophotometer (DR 2000, Loveland, USA) and converted to chlorophyll a concentrations using the equations of SCOR/UNESCO (1966).

Table 1 Means ± SE of physicochemical parameters in the pre-treatment period of "Bti and community" and "community density" experiments

Parameters	Bti and Community	Community density
TDS (mg/L)	79 ± 6	120 ± 10
pH	7.4 ± 0.2	7.1 ± 0.2
O2 (mg/L)	5.3 ± 0.6	4.6 ± 0.5
Temperature (°C)	29.6 ± 0.7	28.7 ± 0.4
Chl a (mg/m^3)	64 ± 9	125 ± 17
Suface area (m^2)	0.63 ± 0.1	0.67 ± 0.1
Volume (L)	1053 ± 172	1028 ± 181

We found no significant differences between setups about physicochemical parameters, in the community experiment as well as in the community density experiment (ANOVA, $P > 0.05$). Indications of the mean values of the measurements realized just before VectoBac treatment are given in Table 1.

Data analysis

All data were subjected to log (x+1) transformation prior to all analyses. One-way ANOVA was used to test for the difference in the abundances of mosquito larvae between setups. The factor treatment type was used to compare the setups "com", "Bti", and "comBti" in the "Bti and community" experiment; and the community density was used to compare the setups "low", "medium", and "high" in the "community density" experiment. A significant ANOVA was followed by a *Bonferroni* post hoc test for paired wise comparisons in the "Bti and community" experiment, and by a *Dunnett-T* post hoc test for paired wise comparisons between the setup "low" and the setups "medium" and "high" in the "community density" experiment.

The Redundancy Analysis (RDA) was carried out on data collected during the recolonisation period to highlight the influence of the factors community density and VectoBac on the structure of the communities of the different setups. RDA is a linear constrained multivariate ordination which is appropriate to describe correlations between response variables and predictors in a complex system characterized by many species (Leps & Smilauer, 2003). For this analysis, the community density and VectoBac were used as predictors, and mosquito larvae and their associated

invertebrate taxa were used as response variables. When a significant correlation was detected, the percentage of explained variations by the first ordination axis was given in brackets on the ordination graph. The percentages of explained variations were obtained by multiplying the fit of each species (provided in the results file of RDA) into the ordination space by 100.0 (ter Braak & Smilauer, 2002).

RESULTS

The outcomes were analysed following three periods of time divided similarly in both experiments. The first period is the pre-treatment that describes an overview about the abundances and the distribution of mosquito larvae in the different setups before the application of VectoBac. The second period is the 24-h post exposure that shows the impact of VectoBac on larval abundances of mosquitoes after one day following the application of VectoBac. The third period is the recolonisation period that lasts from the 24-h post exposure to the end of the experiment; this shows the re-establishment of larval populations of mosquitoes in ponds after the treatment of ponds with VectoBac in relation with the density of invertebrate communities associated. The two mosquitoes identified in the present investigation were *Anopheles* and *Culex* species.

1- "Bti and Community" experiment

1.1- Pre-treatment period

During the pre treatment period, larval abundances of *Anopheles* spp. ("a" in Fig. 1A) and *Culex* spp. ("a" in Fig. 1B) were similar between the setups "com", "Bti", and "ComBti" (ANOVA, $P > 0.05$), as before the treatment no difference was to be expected.

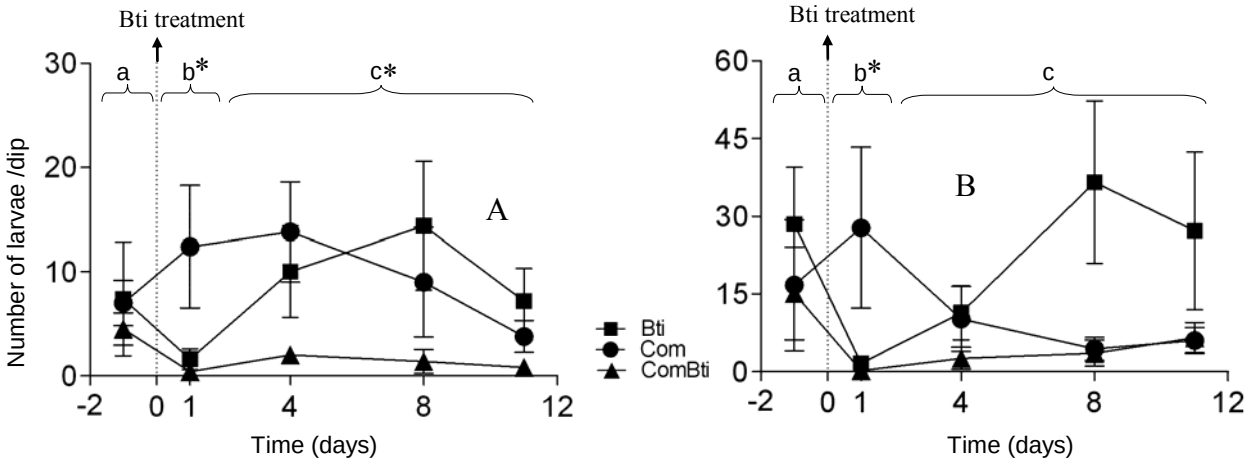


Figure 1. Variations of larval abundances of *Anopheles* spp. (A) and *Culex* spp. (B) in the different setups (i.e. VectoBac alone ("Bti"), community alone ("com"), and "Bti" + "comBti") of the "Bti and community" experiment. "a", "b", and "c" represent the pre treatment, the 24-h post exposure and the recolonisation periods, respectively. " " shows the exposure time of ponds to VectoBac.

1.2- Twenty four hour-post exposure period

In the setups "Bti" (VectoBac only) and "ComBti" (VectoBac + added community), there was a fast reduction of larval abundances of *Anopheles* spp. ("b" in Fig. 1A) and *Culex* spp. ("b" in Fig. 1B) 24 h following the treatment with VectoBac.

Larval abundances of larvae recorded in the pre-treatment period in "Bti" and "comBti" setups, *Anopheles* spp. were reduced to 78 and 91%, respectively; and *Culex* spp. were reduced to 94 and 99%, respectively. By contrast in the setup "com" (added community only), there was an initial increase in larval abundances of *Anopheles* spp. (Fig. 1A) and *Culex* spp. (Fig. 1B) till day 4; this increase was followed by a progressive decrease. The abundances of larvae were significantly higher in the "com" setup than in the "Bti" and "ComBti" setups for *Anopheles* spp. ("b" in Fig. 1A, ANOVA: P = 0.034), as well as for *Culex* spp. ("b" in Fig. 1B, ANOVA: P = 0.002).

These results show that, in contrast to the treatment with added community, the larval abundance of mosquitoes was affected strongly by VectoBac within a short time (i.e. in one day).

1.3- Recolonisation period

In the "Bti" setup, larval populations of *Anopheles* ("c" in Fig. 1A) and *Culex* ("c" in Fig. 1B) showed a good recovery with abundances in the recolonisation period around those recorded in the pre-treatment. In the "comBti" setup, no recovery was observed during the whole recolonisation period for *Anopheles* ("c" in Fig. 1A) and *Culex* ("c" in Fig. 1B). In the "com" setup, the period from day 4 to day 11 was characterised by a progressive decrease in the abundances of larvae of *Anopheles* spp. ("c" in Fig. 1A) and *Culex* spp. ("c" in Fig. 1B).

The only significant difference in larval abundance was found for *Anopheles* spp. between "Bti" and "comBti" setups ("c" in Fig. 1A; ANOVA: P = 0.029, *Bonferroni* post hoc test: P = 0.032). Concerning *Culex* spp., the larval abundance was higher in the setup "Bti" than in the setups "com" and "comBti" during the

recolonisation period ("c" in Fig. 1B); but this difference was not significant (ANOVA: $P > 0.05$).

We carried out a redundancy analysis (RDA) to investigate the influence of the density and the composition of invertebrate communities on the recolonisation of ponds by mosquitoes after VectoBac treatment. The abundances of taxa that were part of the community associated to mosquito larvae are shown in Table 2.

Table 2 Mean and median values of the abundances (individual /L) of invertebrate taxa associated to mosquito larvae in "Bti and community" and "community density" experiments for the whole period of study.

	Bti and community		Community density	
	Mean ± SE	Median	Mean ± SE	Median
Ceriodaphnia spp.	14 ± 5	3	25 ± 7	5
Chydorus spp.	3 ± 0.4	2	0.1 ± 0.1	0
Ciliata	16 ± 2	12	10 ± 1	8
Collembola	34 ± 2	1	3 ± 1	1
Cyclopoida	42 ± 4	36	38 ± 6	19
Hydra sp.	0.2 ± 0.1	0	0.4 ± 0.2	0
Hydracarina	0.2 ± 0.02	0	0.3 ± 0.1	0
L Chironomidae	8 ± 2	3	8 ± 2	3
L Dytiscidae	0.2 ± 0.1	0	0.2 ± 0.1	0
L Ephemeroptera	28 ± 3	24	33 ± 3	26
L Hydrophlilidae	0.2 ± 0.1	0	0.2 ± 0.1	0
L Odonata	4 ± 2	1	4 ± 1	3
Nauplii	13 ± 2	7	10 ± 4	3
Notonectidae	1.2 ± 0.2	1	2 ± 0.2	1
Ostracoda	4 ± 1	2	5 ± 1	1
Planaria	0.2 ± 0.1	0	0.2 ± 0.04	0
Rotifera	38 ± 16	1	100 ± 34	1

"L" = larvae

For the RDA, we used the data of "Bti" and "comBti" setups about the recolonisation period. The predictor was the factor "community density" with two levels; low density was affected to "Bti" and high density to "comBti". The results showed that, invertebrate taxa which were correlated with the first ordination axis had at least 22.5% of their variability associated with the factor community density (Fig. 2, Monte Carlo permutation test: P = 0.032).

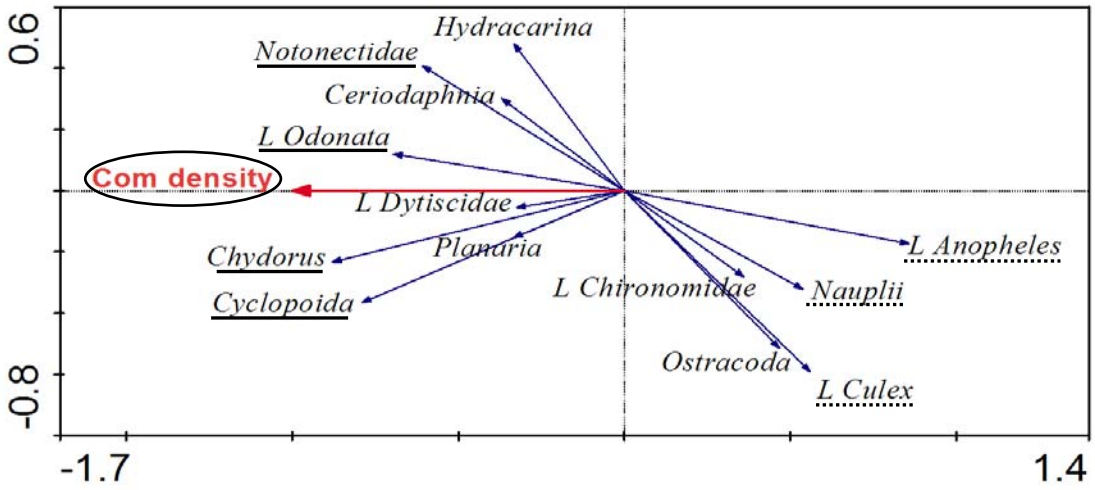


Figure 2. Ordination plot derived by the redundancy analysis (RDA) and showing the community structure of invertebrate taxa in relation to the factor community density (encircled) after VectoBac treatment in the "Bti and community" experiment. Underlined with a continuous line means significant and positive correlation with the community density and underlined with a dashed line means significant and negative correlation with the community density. The percentages in parentheses represent the part of the variations associated to the community density for individual taxa. Only taxa which showed more than 10% of the associated variability with the first ordination axis are exhibited on this graph. "Com density" = Community density and "L" = larvae.

Of these taxa, larvae of *Anopheles*, larvae of *Culex,* and nauplii were negatively correlated with the factor community density whereas *Chydorus* spp., Cyclopoida, Notonectidae, and larvae of Odonata were positively correlated with that factor (Fig. 2). *Chydorus* sp., Cyclopoida, Notonectidae and larvae of Odonata were the influential enemies of mosquito larvae in the "Bti and community" experiment.

In addition, we carried out a RDA to investigate the effects of VectoBac on the community structure during the post exposure period. For this analysis, we used the dataset of the setups "com" and "comBti" about the recolonisation period. The factor "VectoBac treatment" was the predictor here with two levels, absence of VectoBac treatment for the "com" setup and presence for the "Bti" setup. The results showed no significant effects of VectoBac treatment on the community structure of ponds (data not shown, Monte Carlo permutation tests: P = 0.7). This shows that Bti-based VectoBac did not change the community composition of ponds at the concentration of 4 g/m^3 in the study area.

2- "Community density" experiment

2.1- Pre-treatment

Larval abundance in the setup "high" community density was the lowest from the three setups ("low", "medium", and "high" community density) for both mosquito species ("a" in Fig. 3A & 3B). Larval abundance in the setup "medium" community density were intermediate to the two others setups for *Culex* spp. ("a" in Fig. 3B). However, none of these differences were significant (ANOVA, P > 0.05). This is most likely due to the fact that the pre-treatment time was not long enough (three days) to

observe an amplified influence of the community density on the colonisation of ponds by mosquitoes.

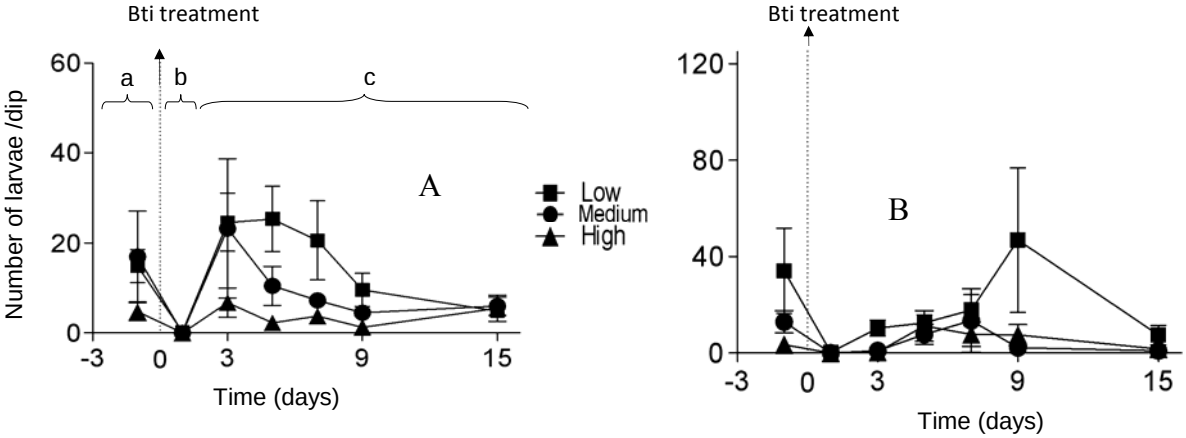


Figure 3. Variations of larval abundances of *Anopheles* spp. (A) and *Culex* spp. (B) in the "community density" experiment. "Low", "medium" and "high" express the different community densities applied in each setup. "a", "b", and "c" represent the pre treatment, the 24-h post exposure and the recolonisation periods, respectively. "→" shows the exposure time of ponds to VectoBac.

2.2- Twenty four hour-post exposure

After 24 h following the treatment of ponds with VectoBac, there was a complete eradication of larvae of *Anopheles* spp. ("b" in Fig. 3A) and *Culex* spp. ("b" in Fig. 3B) from all setups. This result confirms the efficiency of VectoBac observed in the "Bti and community" experiment about controlling larval abundances of mosquitoes in a short time (i.e. in one day).

2.3- Recolonisation period

The recolonisation period was characterized by different patterns in the variations of larval abundance of mosquitoes between the setups “low”, “medium”, and “high” community density (“c” in Fig. 3).

After three days following the treatment with VectoBac, larvae of *Anopheles* spp. had recolonised in the setups “low” and “medium” community density (“c” in Fig. 3A). In the setup “low” community density, a relatively high abundance of larvae of *Anopheles* spp. was maintained till day 7 and was followed by a progressive decrease till the end of the experiment (“c” in Fig. 3A). In the setup “medium” community density, the abundance of larvae of *Anopheles* spp. decreased already from day 5 (“c” in Fig. 3A). In the setup “high” community density, the abundance of larvae of *Anopheles* spp. remained the lowest during the whole post treatment period. The differences in larval abundance of *Anopheles* spp. between the setups “low” and “high” community density were significant (“c” in Fig. 3A; ANOVA: $P = 0.01$, *Dunnet-T* post hoc test: $P = 0.003$).

About *Culex* spp., the abundance of larvae were in general higher in the setup “low” community density than in the setups “medium” and “high” community density, although not significant (ANOVA: $P > 0.05$) (“c” in Fig. 3B). When compared to *Anopheles* spp., the populations of *Culex* spp. were affected to a lesser extent by the community density.

We carried out a RDA to investigate the influence structure of the density and the composition of invertebrate communities on the recolonisation of ponds by mosquitoes after VectoBac treatment. The abundances of taxa that were part of the community associated to mosquito larvae are shown in Table 2.

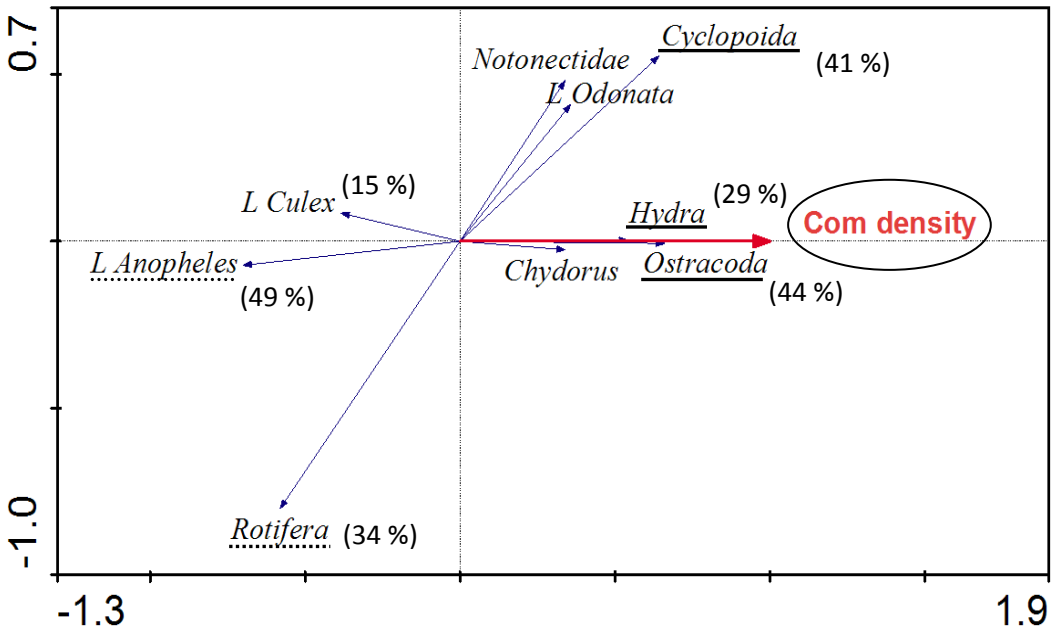


Figure 4. Ordination plot derived by the redundancy analysis (RDA) and showing the community structure of invertebrate taxa in relation to the factor community density (encircled) after VectoBac treatment in the "community density" experiment. The data of all three setups were used for this analysis. Underlined with a continuous line means significant and positive correlation with the community density and underlined with a dashed line means significant and negative correlation with the community density. The percentages in parentheses nearby significant correlations represent the part of the variations associated to the community density for individual taxa. Only taxa which show more than 10% of the associated variability with the first ordination axis are exhibited on this graph. "Com density" = Community density and "L" = larvae.

We used the data of the "low", "medium", and "high" setups about the recolonisation period. The factor "community density" was the predictor with three levels, i.e. low, medium, and high community density. The outcomes revealed that the

invertebrate taxa which were correlated with the first ordination axis had at least 22.8% of their variations associated to the factor "community density" (Fig. 4; Monte Carlo permutation test: P = 0.012). Of these taxa, larvae of *Anopheles* spp. and Rotifera were negatively correlated with the factor community density whereas species of Cyclopoida, *Hydra* sp. and Ostracoda showed positive correlations with the community density (Fig. 4). Cyclopoida, *Hydra*, and Ostracoda were the influential enemies of mosquito larvae in the "community density" experiment. The other invertebrate taxa did not show significant correlations with the community density (Fig 4). However, the association between the abundance of larvae of *Culex* spp. and the factor "community density" was negative (Fig 4).

These results suggest that, the recolonisation of ponds by mosquito populations following the treatment with VectoBac was strongly negatively affected by the highest density of the community.

In both experiments, *Anopheles* spp. was more affected by the factor community density than *Culex* spp. (Fig. 2 & 4, respectively). This outcome suggests that the population of *Anopheles* spp. might be more influenced by a high community density than *Culex* spp.

DISCUSSION

Liess and Duquesne (2009) have defined a two-step method for larval control of pests in water bodies. One step is the suppression of pest larvae using an insecticide,

and another step is the prevention of the repopulation using biological agents such as competitors for food of the pest larvae. In the present study, we implement this approach and offered in addition practical facilities for field operations.

Contribution of the present results to mosquito control

We found that the use of VectoBac in combination with added community was more efficient for larval control of mosquitoes than the use of community alone, or VectoBac alone. The efficiency of this combined treatment increased with increasing density of invertebrate community represented in this study by potential competitors such as Ostracoda and *Chydorus* spp., and predators such as Cyclopoida, *Hydra* sp., Notonectidae and larvae of Odonata.

The reinforcement of a Bti-based control of mosquito larvae (e.g. *Aedes aegypti*) by an invertebrate species (e.g. the Cyclopoida *Mesocyclops aspernicornis*) was shown in water storage containers in Thailand (Kosiyachinda et al. 2003). The use of a specific organism requires among other qualifications, a good knowledge of the taxonomic classification of that organism. Moreover, this particular species must be cultured successfully and survive the transfer to the field. However, collecting invertebrate communities from older ponds, as we did in our study, has the advantage that no scientific background is required. In comparison to the culture, the method of organism collection proposed in the present study is cost effective and also time and energy saving. The chance of success is higher in this case because taxa are adapted to the environmental conditions of the area to be treated. So, our practice should be more easily implemented by neighbouring and non expert inhabitants of zones at high incidence of mosquito problems such as Mfou, Cameroon.

In tropical regions in general and in Cameroon in particular, temporary water bodies are generated spontaneously in the ground depressions in the onset of rainfalls. These ponds serve as additional breeding sites during the rainy seasons (Chave and Koeenradt 2010). Studies have shown that natural enemies (i.e. predators and competitors) can affect the abundances of larval populations of mosquitoes (Meyabeme et al. 2010); causing direct lethal effects on mosquito larvae (Knight et al. 2004, Marten & Reid 2007, Duquesne et al. 2010) or/and deterring oviposition by gravid females (Stav et al. 2000, Eitam et al. 2002, Mokany & Shine 2003, Blaustein et al. 2004, Duquesne et al. 2010). Newly flooded ponds shelter low abundances of competitors and predators (Wilbur 1997). Opportunistic organisms like mosquitoes exploit preferentially such conditions to maximize the development success of their offspring (Mokany & Shine 2003, Blaustein et al. 2004, Duquesne et al. 2010). This might explain the outbreaks of mosquito populations observed in tropical regions at the beginning of the rainy seasons (Bigoga et al. 2007, Atangana et al. 2009). This study proposes a strategy that can deal with those outbreaks, i.e. suppression of mosquito larvae using a Bti-biopesticide (e.g. VectoBac) and anticipating the ageing of food webs of newly flooded ponds to prevent high abundances of mosquito larvae after treatment.

Anopheles and *Culex* species: susceptibility to the treatments with VectoBac and high community densities

The larval populations of the two mosquitoes drastically decreased in all VectoBac treatments within 24 h after treatment. This efficacy of the VectoBac formulation of Bti has also been reported in other countries such as India (Amalraj et

al. 2000), Kenya (Kahindi et al. 2008), Australia (Russel et al. 2003), and Turkey (Aldemir 2007). Owing to the fact that larval development of mosquitoes can last only few days (e.g. seven days, Mokany & Shine 2003), fast acting larvicides such as Bti should be integrated in mosquito control strategies.

Anopheles species were affected more strongly by high community density than *Culex* species. It was revealed that some of the predators that had a positive correlation with the community density (e.g. Cyclopoida) during the present study preyed substantially more on larvae of *Anopheles* than on larvae of *Culex* (Marten & Reid 2007). Therefore, the higher susceptibility of populations of *Anopheles* may be a consequence of prey preference behaviour by certain predators. Although we could not explicitly separate the relevance of the direct lethal effect by natural enemies on mosquito larvae from the oviposition deterring behaviour of female mosquitoes, the expected role of invertebrate community in preventing the recolonisation of ponds by mosquitoes after a treatment with VectoBac was proved in this study.

CONCLUSION

Our results suggest that natural invertebrate communities had a great potential in sustaining the larval control of mosquitoes by Bti-based VectoBac. So, owing to the fact that newly flooded ponds contribute in increasing the abundance of the disease vector mosquitoes, we recommend the combination of VectoBac and local invertebrate communities for larval control of this pest, especially during the first weeks of rainy seasons.

ACKNOWLEDGMENTS

We would like to thank K. Foit for helping with statistical analysis, T. Njine, C.F. Bilong Bilong, S. Zebaze Togouet for their active participation in the establishment of the collaboration between the Helmholtz Centre for Environmental Research-UFZ Leipzig (Germany) and the laboratory of General Biology of the University of Yaounde I (Cameroon). We are grateful to P. Ywuoung, R. Kayo, J. Nzieleu, H. Tsila, and N.F. Meyong for field assistance; and inhabitants of Mfou arrondissement for their enthusiastic adoption of this project. This work was supported financially by DAAD (German Academic Exchange Service) and the Helmholtz Centre for Environmental Research-UFZ Leipzig, Germany.

REFERENCES

Aldemir A, 2007. The Efficacy and longevity of Vectobac® 12 AS and Vectobac® G (both based on *Bacillus thuringiensis* subsp. *israelensis*) for the control of mosquitoes in Turkey. *Turk J Zool 31:* 317--323.

Amalraj DD, Sahu SS, Jambulingam P, Boopathi Doss PS, Kalyanasundaram M, Das PK, 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis (Vectobac)* against mosquito vectors. *Acta Tropica 75:* 243--246.

Atangana J, Fondjo E Fomena A, Tamesse J L, Patchoké S, Ndjemaï HNM, Bessong Ndong PA, 2009. Seasonal variations of malaria transmission in Western

Cameroon highlands: Entomological, parasitological and clinical investigations. *Journal of Cell and Animal Biology 3 (3):* 033--038.

Barbazan P, Baldet T, Darriet F, Escaffre H, Haman Djoda D, Hougard J-M, 1997. Control of Culex quinquefasciatus (Diptera: Culicidae) with Bacillus sphaericus in Maroua, Cameroon. *J Am Mosq Control Assoc 13 (3):* 263--269.

Becker N, Petrić D, Zgomba M, Boase C, Dahl C, Lane J, Kaiser A, 2003. Mosquitoes and their control. New York, NY: Kluwert Academic/Plenum Publishers, 1-451.

Bigoga JD, Manga L, Titanji VPK, Coetzee M, Leke RGF, 2007. Malaria vectors and transmission dynamics in coastal south-western Cameroon. *Malar J 6:* 5--17.

Blaustein L, Kiflawi M, Eitam A, Mangel M, Cohen JE, 2004. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia 138:* 300--305.

Boisvert M, 2005. Utilization of Bacillus thuringiensis var. israelensis (Bti)-based formulations for the biological control of mosquitoes in Canada. 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact. J.-C. Côté, I. S. Otvos, I.S., J.-L. Schwartz & C. Vincent. Victoria, BC.

Boisvert M, Boisvert J, 2000. Effect of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. *Biocontrol Sci Technol 10:* 517--561.

Camargo EP, 2008. Tropical diseases. *Estudos Avançados 22 (64):* 95-110.

Chaves LF, Koenraadt CJM, 2010. Climate change and highland malaria: fresh air for a hot debate. *Q Rev Biol 85 (1):* 27--55.

Duquesne S, Kroeger I, Kutyniok M, Liess M, 2010. Competiting zooplankton communities reduce development of larval populations of mosquitoes. *J Med Entomol (in press)*.

Durand JR, Lévêque C, 1980. Aquatic flora and fauna of sahelo-Sudanese Africa, Volume I. Paris: Office for Overseas Scientific and Technical Research (O.R.S.T.O.M), 152-368.

Eitam A, Blaustein L, Mangel M, 2002. Effects of *Anisops sardea* (Hemiptera : Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia 485:* 183--189.

Faye O, Konate L, Mouchet J, Fontenille D, Ngayo Sy S, Hebrard G, Herve JP, 1997. Indoor resting by outdoor biting females of *Anopheles gumbiue* complex (Diptera: Culicidae) in the Sahel of Northern Senegal. *J Med Entomol 34 (3):* 285--289.

Fontenille D, Carnevale P, 2006. Malaria and dengue vector biology and control in West and Central Africa. Knols BGJ, Louis C, eds. Bridging laboratory and field research for genetic control of disease vectors. Dordrecht: Springer, 91-99.

Hemingway J, Ranson H, 2000. Insecticide resistance in insect vectors of human disease. *Ann Rev Entomol 45:* 371--391.

Hougard J-M, Back C, 1992. Perspective on the bacterial control of Vectors in the tropics. *Parasitol Today 8 (11):* 364--366.

Kahindi SC, Midega JT, Mwangangi JM, Kibe LW, Nzovu J, Luethy P, Githure J, Mbogo C M, 2008. Efficacy of Vectobac dt and CulinexCombi against

mosquito larvae in unused swimming pools in Malindi, Kenya. *J Am Mosq control Assoc. 24 (4):* 538--542.

Knight TM, Chase JM, Goss CW, Knight JJ, 2004. Effects of interspecific competition, predation and the interaction on survival and development time of immature *Anopheles quadrimaculatus*. *J Vector Ecol 29 (2):* 277--284.

Kosiyachinda P, Bhumiratana A, Kittayapong P, 2003. Enhancement of the efficacy of a combination of *Mesocyclops aspericornis* and *Bacillus thuringiensis* var. *israelensis* by community-based products in controlling *Aedes aegypti* larvae in Thailand. *Am J Trop Med Hyg 69(2):* 206--212.

Kumar R, Hwang JS, 2006. Larvicidal efficiency of aquatic predators: a perspective for mosquito biocontrol. *Zool stud 45 (4):* 447--466.

Lacey, L. A. 2007. *Bacillus thuringiensis serovariety israelensis* and *Bacillus sphaericus* for mosquito control. *J Am Mosq Control Assoc 23 (2):* 133--163.

Liess M, Duquesne S, 2010. Method for the eradication of pathogenic microorganisms in an aqueous system. Patent PTC/EP2009/065025, filed November 12, 2009, *(in pending)*.

Leps J, Smilauer P, 2003. Multivariate analysis of ecological data using CANOCO. Cambridge: Cambridge University Press, 38-43.

Marten GG, Reid JW, 2007. Cyclopoid copepods. *J Am Mosq Control Asso 23:* 65--92.

Mokany A, Shine R, 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. *Austral Ecology 28:* 33--37.

Meyabeme Elono AL, Liess M, Duquesne S, 2010. Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetlands areas in Germany. *J Vector Ecol (in press).*

Mutuku F, Alaii MJA, Bayoh MN, Gimnig JE, Vulule JM, Walker ED, kabiru E, Hawley WA, 2006. Distribution, description, and local knowledge of larval habitats of *Anopheles gambiae* s.l. in a village in Western Kenya. *Am J Trop Med Hyg 74 (1):* 44--53.

Oyewolea IO, Awololab TS, Ibidapoc CA, Oduolad AO, Okwac OO, Obansad JA, 2007. Behaviour and population dynamics of the major anopheline vectors in a malaria endemic area in southern Nigeria. *J Vect Borne Dis 44:* 56--64.

Ramirez JL, Garver LS, Dimopoulos G, 2009. Challenges and Approaches for mosquito targeted malaria control. *Curr Mol Med 9:* 116--130.

Ross, R. 1897. On some peculiar pigmented cells found in two mosquitos fed on malarial blood. *Br Med J 2 (1929):* 1786-1788.

Russell TL, Brown MD, Purdie DM, Ryan PA, Kay BH, 2003. Efficacy of VectoBac (*Bacillus thuringiensis* variety *israelensis*) formulations for mosquito control in Australia. *J Econ Entomol 96 (6):* 1786--1791.

Schwab H, 1995. Freshwater animal. Stuttgart: Klett Schubuch, 246-351.

SCOR/UNESCO 1996. Determination of Photosynthetic Pigments in Seawater. Paris: UNESCO, 11-18.

Simard F, Ayala D, Kamdem GC, Pombi M, Etouna J, Ose K, Fotsing J-M, Fontenille D, Besansky NJ, Costantini C, 2009. Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. *BMC Ecology 9:*17.

Stav G, Blaustein L, Margalit J, 2000. Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito *Culiseta Longiareolata* (Diptera:Culicidae) and community structure in temporary pools. *J Vector Ecol 25:* 190--202.

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P, 2003. Freshwater invetebrates, systematic, biology, ecology. Paris: CNRS, 62-351.

ter Braak CJF Smilauer P, 2003. Canoco for Windows Version 4.52. Wageningen: Biometrics-Plant Research International, 174-183.

Tuno Nobuko, Kjaerandsen J, Badu K, Kruppa T, 2010. Blood-feeding behaviour of *Anopheles gambiae* and *Anopheles melas* in Ghana, Western Africa. *J Med Entomol 47 (1):* 28--31.

Ward HB, Whipple GC, 1959. Fresh-water biology. New York, London: John Wiley & Sons Inc, 265-858.

WHO [World Health Organization], 1975. Manual on practical entomology in Malaria. Part II: methods and techniques. Geneva: World Health Organization Offset Publication No. 13, 55-60.

Wilbur HM 1997. Experimental ecology of food webs: complex systems in temporary ponds. *Ecol 78(8):* 2279--2302.

Chapter V

General discussion

As described in the general introduction (Chapter I), there is a great need for safe and successful methods in mosquito control. The present thesis reveals the relevance of considering and integrating natural enemies of mosquito larvae (i.e. competitors for food and predators) in control strategies.

The study in Chapter II describes the relationships between mosquito larvae and their associated invertebrate communities in temporary water bodies in the nature. The results showed that larval abundance of mosquitoes in general, and of *Aedes* spp. in particular, was negatively associated with the abundance of competitors for food sources (e.g. cladocera), and to a lesser extent, with the abundances of intraguild predators (e.g. Cyclopoida), and strict predators (e.g. larvae of Odonata). This is well in line with previous works that showed similar relationships between other mosquito genera (e.g. *Anopheles quadrimaculatus* and *Culex pipiens*) and their competing and predatory taxa (Chase & Knight 2003). Theses natural enemies can decrease the survival of mosquito populations by causing direct lethal effects on mosquito larvae (Knight et al. 2004, Marten & Reid 2007) or/and deterring oviposition by gravid females (Eitam et al. 2002, Mokany & Shine 2003, Duquesne et al. 2010).

The impact of these competing and predatory taxa on mosquito larvae is strong but, cannot be as fast and efficient as the impact of insecticides (Chapter 4) that are basically used in case of public health crisis caused by mosquito borne diseases (Tren, 2009). However, from a point of view of environmental safety, numerous studies have

demonstrated side effects of biological and chemical insecticides (Hershey et al 1998, Poulin et al. 2010). Such effects include for example the direct mortality of non target organisms or/and changes of the community structure (Mortimer & Chapman 1995, Clements & Newman 2002). To decrease the amount of insecticides used so that these unwanted damages are minimised, control methods combining insecticides and natural enemies (Liess & Duquesne, 2009) are of a great interest. This makes it possible to kill mosquito larvae using insecticides and prevent their re-establishment in the breeding sites using natural enemies (Liess & Duquesne, 2009).

Biological pesticides based on the bacteria *Bacillus thuringiensis* var *israelensis* (Bti) are considered to be among the safest for mosquito control (Boisvert 2005). Bti is very fast and kills mosquito larvae within 24 hours after exposure (Amalraj et al. 2000, Aldemir 2007, Kahindi et al. 2008). Kosiyachinda et al. (2003) prolonged the effect of Bti on the mosquito *Aedes aegyti* using the copepod *Mesocyclops aspericornis*. The selection of a specific organism requires for example a good knowledge of its taxonomic classification; it must be cultured successfully and survive the transfer to the field. In the study in Chapter IV, we enhanced successfully the efficacy of Bti with invertebrate communities collected from older ponds of the same area. In comparison to a culture of a specific natural enemy, no scientific background is needed here; the method is cost effective and also time-efficient. The chances of success are higher in our case because organisms are adapted to the environmental conditions of the area. Thus, our practice might be implemented easily by non expert people who live in areas at high incidence of mosquito problems.

Since thirty years, no new public health insecticide is available for vector control; thus, the development of insecticides with new mode of action was raised

recently as an urgent tool to mitigate mosquito problems (Hemingway et al 2006, Mendis et al.2009). The study in Chapter III presents ecological effects of a relatively new insecticide (i.e. thiacloprid) that shows a promising efficiency against mosquitoes. However, thiacloprid also reduces competing and predatory invertebrates and hence, even supports the development of mosquito larvae. This confirms the competence of natural enemies as regulators of larval populations of mosquitoes discussed in Chapters II and IV. Mosquitoes have a short generation time (e.g. 7 days, Mokany & Shine 2003) and a semi aquatic life cycle, i.e. larvae are aquatic and adults are terrestrial (Becker et al. 2003). When the development of competitors and/or predators depends exclusively on aquatic environment, the recovery of their populations is delayed after pesticide treatment. This release of the predation and competition pressures in a breeding site may create suitable conditions for more oviposition by opportunistic mosquitoes and a better development of their offspring. Therefore, it is important that potential new insecticides for larval control of mosquitoes are particularly non toxic to natural enemies that can prevent secondary outbreaks of mosquito populations.

In sum, the present thesis provides valuable information for integrated management of mosquito control and risk assessment of pesticides in aquatic ecosystems. In a next step, the novel approach of combining biological insecticides and natural enemies have to be validated in the field at a larger scale.

Literature cited Chapter V

Aldemir, A. 2007. The Efficacy and longevity of Vectobac® 12 AS and Vectobac® G (both based on *Bacillus thuringiensis* subsp. *israelensis*) for the control of mosquitoes in Turkey. Turk J Zool 31: 317-323.

Amalraj, D. D, S.S. Sahu, P. Jambulingam, P.S. Boopathi Doss, M. Kalyanasundaram, & P.K. Das. 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis (Vectobac)* against mosquito vectors. Acta Tropica 75: 243-246.

Becker, N., D. Petrić, M. Zgomba, C. Boase, C. Dahl, J. Lane, & A. Kaiser. 2003. *Mosquitoes and their control*. Kluwert Academic/Plenum Publishers, New York, N.Y., USA.

Boisvert, M. 2005. Utilization of *Bacillus thuringiensis* var. *israelensis* (Bti)-based formulations for the biological control of mosquitoes in Canada. 6th Pacific Rim Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact. J.-C. Côté, I. S. Otvos, I.S., J.-L. Schwartz & C. Vincent. Victoria, BC.

Chase, J.M. & T.M. Knight. 2003. Drought induced mosquito outbreaks in wetlands. Ecol Lett 6: 1017-1024.

Clements, H.W. & M.C. Newman. 2002. *Community ecotoxicology*. John Wiley & Sons, West Sussex, UK.

Duquesne, S., I. Kroeger, M. Kutyniok, and M. Liess. 2010. The potential of Cladocera as controphic competitors of the mosquito *Culex pipiens*. J Med Entomol (in press).

Eitam, A., L. Blaustein, & M. Mangel. 2002. Effects of *Anisops sardea* (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. Hydrobiologia 485: 183-189.

Hemingway, J., B.J. Beaty, M. Rowland, T.W. Scott, & B.L. Sharp. 2006. The innovative vector control consortium: improved control of mosquito-borne diseases. Parasitol today 22 (7): 308-312.

Hershey, A.E., A.R. Lima, G.J. Niemi, & R.R. Regal. 1998. Effects of Bacillus thuringiensis israelensis (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecol Appl 8 (1): 41-60.

Kahindi, S. C., J. T. Midega, J. M. Mwangangi, L. W. Kibe, J. Nzovu, P. Luethy, J. Githure & C. M. Mbogo. 2008. Efficacy of Vectobac dt and CulinexCombi against mosquito larvae in unused swimming pools in Malindi, Kenya. J Am Mosq Control Assoc 24 (4): 538-542.

Knight, T.M., J.M. Chase, C.W. Goss, & J.J. Knight. 2004. Effects of interspecific competition, predation and the interaction on survival and development time of immature *Anopheles quadrimaculatus*. J Vector Ecol 29(2): 277-284.

Kosiyachinda, P., A. Bhumiratana, & P. Kittayapong 2003. Enhancement of the efficacy of a combination of *Mesocyclops aspericornis* and *Bacillus thuringiensis* var. *israelensis* by community-based products in controlling *Aedes aegypti* larvae in Thailand. Am J Trop Med Hyg 69(2): 206–212.

Liess, M. & S. Duquesne 2009. Method for the eradication of pathogenic microorganisms in an aqueous system. Patent PTC/EP2009/065025, filed November 12, 2009, (in pending).

Marten, G.G. & J.W. Reid. 2007. Cyclopoid copepods. J Am Mosq Control Assoc 23: 65-92.

Mendis, K., A. Rietveld, M. Warsame, A. Bosman, B. Greenwood, & W.H. Wernsdorfer. 2009. From malaria control to eradication: the WHO perspective. Trop Med Int Health 14 (7): 802-809.

Mokany, A. & R. Shine. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecology 28: 33–37.

Mortimer, M.R. & H.F. Chapman. 1995. Acute toxic effects of (S)-methoprene and temephos to some Australian non-target aquatic crustacean species. Aust J Ecotoxicol 1: 107-111.

Poulin, B., G. Lefebvre, & L. Paz. 2010. Red flag for green spray: adverse trophic effects of Bti on breeding birds. J Appl Ecol 47: 884-889.

Tren, R. 2009. Insecticides for public health. CMAJ 180 (7): 784.

Declaration on the contributions to the manuscripts/papers on which this thesis is based:

Meyabeme Elono, A.L., M. Liess, and S. Duquesne. 2010. Influence of competing and predatory invertebrate tax on larval populations of mosquitoes in temporary ponds of wetland areas in Germany. J Vector Ecol (*in press*).

Own contribution: Field work, statistical analysis of data, results interpretation, manuscript writing and preparation.

Meyabeme Elono AL, Duquesne S, Foit K, Liess M. Population response of *Culex pipiens* to thiacloprid is altered by interspecific interactions. Ecotoxicology (*in preparation for submission*).

Own contribution: Field work, statistical analysis of data, results interpretation, manuscript writing and preparation.

Meyabeme Elono AL, Liess M, Duquesne S. Invertebrate density sustaining the efficiency of Bti based VectoBac in mosquito control, a case study in Cameroon. Am J Trop Med Hyg (*in preparation for submission*).

Own contribution: Field work, statistical analysis of data, results interpretation, manuscript writing and preparation.

CURRICULUM VITAE

Personal data

Surname: Meyabeme Elono

First name: Alvine Larissa

Born on: 29.06.1975 in Yaounde

Address: Strasse des 18. Oktober 31, 233

D-04103 Leipzig

Tel. +49 (0) 162 329 725 4

E-mail: meyabemealvine@yahoo.fr

Education

Since 04/2007	PhD student, with a scholarship of the German Academic Exchange Service (DAAD), at the Helmholtz Centre for environmental research-UFZ, Leipzig, in the department of System Ecotoxicology and in collaboration with the Department of Zoology of Martin-Luther-Universität Halle-Wittenberg.
2001-2003	Master's with thesis (DEA, thesis grade: *Very good*) in Hydrobiology and quality of surface waters at the University of Yaounde I, Cameroon.
2000-2001	Master's degree without thesis (Maitrise) in Hydrobiology and quality of surface waters at the University of Yaounde I, Cameroon.
1996-2000	Bachelor's degree (Licence) in Animal Biology at theUniversity of Yaounde I, Cameroon.

1989-1996	Higher leaving certificate (BACCALAUREAT D) in Natural Sciences and Mathematics at Modern and Classical Secondary School of Sangmelima, Cameroon.
1982-1989	First leaving Certificate (CEP) at Mvan primary School.
1980-1982	Nursery school of Akonolinga, Cameroon.

Awards

2006	PhD scholarship by the German Academic Exchange Service (DAAD)
2003	Financial grant to the best students by the ministry of Higher Education of Cameroon for the finalization of the Master's research work.
2002	Financial grant to the best young scientific girls by the Ministry of Higher Education of Cameroon, session of 2002.
1999	Financial grant to the best young scientific girls by the Ministry of Higher Education of Cameroon, session of 1999.

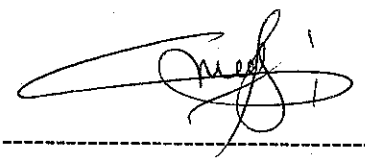
Tutoring activities

2002-2006	Teaching assistant in the Department of Animal biology and physiology of the University of Yaounde I, Cameroon.

CERTIFICATION STATEMENT

I hereby certify that the present dissertation is based on my original work, and was written by me independently and without any unauthorized aids. Authors or works other than those cited in the present thesis were not exploited. I declare that this work has not been previously or concurrently submitted for any other degree to another academic institution in Germany or abroad.

Leipzig, 29th November 2010



Alvine L. Meyabeme E.