12. Classic and novel tools for mosquito control worldwide

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Abstract

Mosquitoes (Culicidae) are at the centre of worldwide entomological research and control efforts primarily because of their medical importance as vectors of diseases, like malaria, dengue, Zika, Chikungunya, West Nile or Yellow fever. They are responsible for more than half a million deaths per year. Despite their role as vectors, culicids can also cause considerable nuisance like floodwater mosquitoes frequently create as they can reproduce in a short time in enormous numbers. The consequence is that outdoor activities in parks or recreation areas are not possible and this has a detrimentrous effect on touristic activities. The most successful approach for managing nuisance or vector mosquitoes is when an integrated vector mosquito management (IVM) is implemented in which all appropriate technologies and control techniques are used, to bring about a decline of target species populations in a cost effective and environmentally safe manner. The IVM strategy can include environmental management, physical, chemical, biological or genetical components. Environmental management means physical reduction of breeding resources, water management to create conditions unfavourable for mosquito breeding. Physical control includes the use of nets and surface layers to avoid vector contact or breathing by mosquito developing stages. Chemical control by using organochlorines, organophosphates, carbamates or pyrethroids is still the most frequently practised approach to combat mosquitoes but usually these chemicals are broad-spectrum products which can have also unwanted side effects on non-target organisms and on the biodiversity when they are used in ecological sensitive areas. Therefore, biological control aiming at the reduction of target populations by the use of predators, pathogens or toxins from microorganisms are nowadays more and more in focus of control operators. Especially the use of protein toxins such as from *Bacillus thuringiensis israelensis* or *Lysinibacillus sphaericus* provide efficient control of target organisms on the one hand and environmental safety on the other hand. The increased application of biological and microbiological methods or Insect growth regulators as well as genetic methods as the Sterile Insect technique (SIT) contribute to an environmentally friendly solution of the mosquito problems. New and improved techniques like the CRISPR (clustered regularly interspaced short palindromic repeats) as a mean of editing mosquito genomes to drive desirable gene constructs into mosquito population can help in future to avoid the transmission of human pathogens. The Geographic Information System (GIS) integrated with digital mobile collection systems supported by a Global Positioning System (GPS) and modern information-technology, can significantly contribute to improving the planning, realisation and documentation of mosquito control/management operations and allow a more effective effort to reducing mosquito-borne diseases. It is out of question that all strategies should involve the public to raise the awareness of people, e.g. for the control of invasive mosquitoes by community participation.

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Introduction

Mosquitoes (Diptera: Culicidae) represent one of the most important family of arthropods in the worldwide transmission of pathogens to humans and animals (Becker *et al.* 2003, Taylor *et al.* 2010), being consequently one of the most important causes of mortality in developing countries. The fight against mosquitoes has ancient origins that date back to the times of Herodotus (V century BC) (Genchi and Pozio 2004) when it was aimed to reduce the nuisance caused by these insects (Swift 2008). Just from the end of the 19th century, with the discovery of the role of vector of heartworms and malarial plasmodium (Capanna 2006, Chernin 1983), the mosquito control strategies begin to take public health connotations. Many countries applied both chemical and mechanical methods, mainly through the use of the organic chlorinated insecticide DDT, and the remediation of marsh areas (Rahman 2013, Tognotti 2008), respectively.

The progressive increase in knowledge about pathogens transmitted by mosquitoes and the spread of alien species introduced in areas far from their origins in recent years, have required even greater attention from public health institutions. At this point, the implementation of mosquito control programs for the prevention of epidemic situations and for the improvement of the quality of life, are considered a priority in many countries (Bellini *et al.* 2011).

Besides the impact of mosquitoes on public health, these insects are also a great threat for wildlife. Many zoonoses caused by viruses and parasites are transmitted by mosquitoes. Avian populations are strongly affected by arbovirus like West Nile, Sindbis or Usutu Virus among many others. These arboviruses are mainly transmitted by *Culex* and *Culiseta* mosquitoes, and despite they have an African origin nowadays outbreaks of these pathogens can be seen on bird populations in different continents due to bird migration processes and climate change conditions which favours virus replication in local mosquito populations (Buckley *et al*. 2003, Ling *et al.* 2019, Reusken *et al*. 2011). Avian malaria is a parasitic disease caused by Apicomplexa species belonging to the genera *Plasmodium* and *Hemoproteus* which can be also transmitted by mosquitoes, once again *Culex* and *Culiseta* species as principal vectors due to their ornithophilic behaviour. There is a wide range of symptoms that these parasites can provoke on birds, from asymptomatic infections to serious affectations characterised by fever, diarrhoea, and anaemia. Even in some cases drastic declines on local bird populations have been described associated to avian malaria epizootics (Van Riper *et al.* 1986). From the point of view of mosquito-borne zoonoses that can seriously affect wildlife, Dirofilariasis (basically associated to *Dirofilaria immitis* and *Dirofilaria repens*) and sylvatic Yellow Fever epizootics on non-human primates can be also highlighted.

Mosquito control can be achieved through the application of direct control techniques which affects the target organisms increasing their mortality, or alternatively through indirect control techniques which affects their reproduction rate (Figure 1).

The mosquito prevention and control techniques can be classified by the following typologies according to their effects, approaches and origin of the control tools (Table 1):

Environmental. It includes indirect control methods which predominantly try to make a specific vulnerable territory (e.g. crop area with permanent stagnant water) unsuitable for the development of mosquitoes, after the application of several environmental changes which, when linked to the

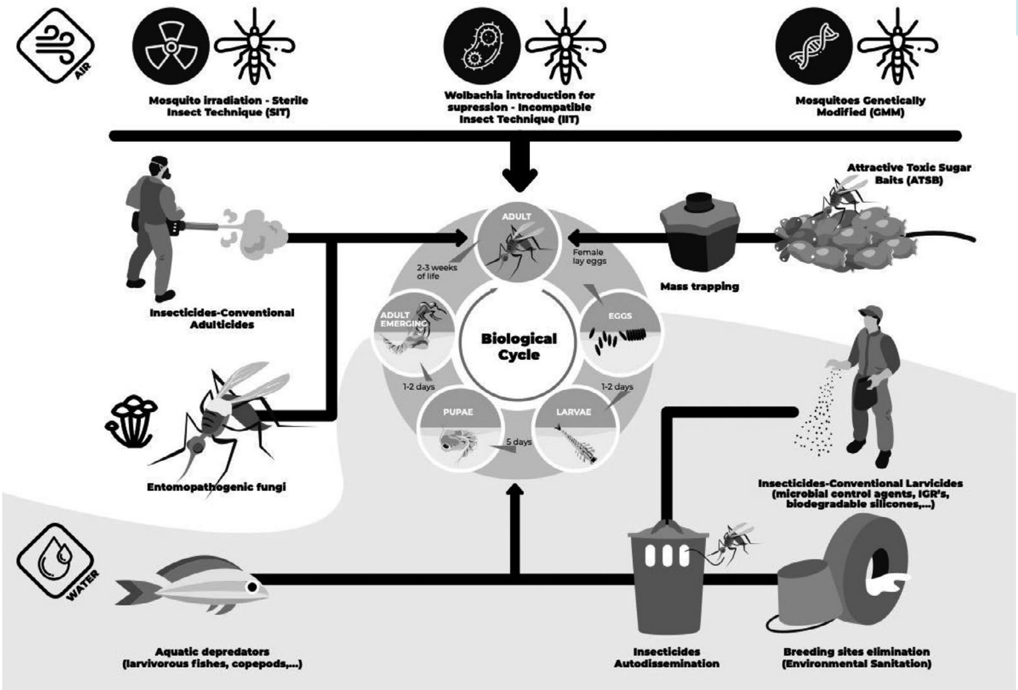

Figure 1. State of the art of different mosquito control strategies, specifying their impact on adult or juvenile life stages.

Table 1. Prevalent effect of different mosquito control strategies.[1]

Type of struggle	Mortality increasing	Reduction of reproduction rate	Feeding suppression
Environmental	–	+	–
Physical	–	–	+
Mechanical	–	+	+
Biological	+	–	–
Biotechnology	–	+	–
Chemical	+	–	+
Educational	+	+	–
Legislative	+	+	–

[1] Positive effect (+), non-effect (–).

cultivation cycles, are named 'agronomical'. A typical example of environmental control strategy is the one carried out in Italy in the early decades of the 1900s to suppress the proliferation of *Anopheles* Meigen, 1818 (Majori and Napolitani, 2010). The reduction of the potential breeding sites are included in the environmental method (Dutto *et al.,* 2012).

Physical. It is an indirect method that aimed to transform habitats potentially colonised by mosquitoes in non-suitable ones through the modification of environmental conditions.

Mechanical. It probably represents the oldest mosquito fighting strategy, essentially based in the interposition of physic barriers (e.g. mosquito nets) that prevent the access of mosquitoes to oviposition sites, or host for blood feeding. Subsequently, substances capable of blocking the gaseous exchanges of the larvae and pupae with the atmosphere (e.g. silicones, oils) have been developed in last decades, provoking asphyxiation processes in immature aquatic stages.

Biological. It involves the use of natural predators (e.g. fishes, copepods), microorganisms (e.g. bacteria, fungi) or toxins linked to these microorganisms.

Biotechnology. The strong advances of molecular manipulation techniques has allowed the use of genetically modified mosquitoes (GMM), mosquitoes irradiation to achieve sterile insect techniques (SIT) or the introduction of endosymbionts like *Wolbachia* to promote the employment of incompatible insect techniques (IIT). Most of these methods try to suppress mosquito populations reducing their fertility or lifespan.

Chemical. Together with the mechanical methods, chemical approaches represent one of the oldest control methods. Chemical control plays a crucial role in the control of mosquitoes both at the larval and adult stage, and it is essentially based on the dispersion of chemical synthetic substances capable to cause direct lethality or repellency on target insects.

Educational. The engagement of citizens and local communities in the fight against mosquitoes is essential, particularly in domestic habitats where many species of mosquitoes can find adequate breeding sites. Door to door strategies and informative campaigns in towns, residential areas and schools, have been implemented in many countries in order to reduce the infestation levels of *Aedes* Meigen, 1818 mosquitoes which can cause important outbreaks of arboviruses.

Legislative. The adoption of legal regulatory rules that oblige the implementation of individual and collective preventive measures to fight against mosquitoes is currently considered as an important issue in any local mosquito control program.

The joint application of several techniques, in association with appropriate surveillance and monitoring programs, allows the development of integrated mosquito management programs (IMMP) which improve efficacy, efficiency and safety of interventions.

Chemical control

Adulticiding

In an IMMP, adulticidal applications should be performed just in specific situations to complement larvicidal interventions, or also to manage situations of mosquito-borne diseases (MBD) epidemics. The use of the adulticides as unique intervention strategy has been demonstrated ineffective to maintain low levels of mosquitoes in a determined area. Moreover, insecticides used to kill adults (mostly pyrethroids) in outdoors have a high environmental impact (Hénault-Ethier 2015).

The adulticidal fight is purely chemical and is implemented through the environmental dispersion of molecules with killing insecticidal activity and can be carried out both indoors and outdoors. The ideal adulticiding should aim at the treatment of resting sites in the hours of inactivity of the target species and taking into account the favourable environmental conditions of each time. Adulticidal treatments can be divided into (Romi *et al.* 2009):

Spatial treatments in outdoor conditions, generally concerning vegetation of green areas, heaps of materials (e.g. demolition) or ruins of buildings in degraded urban areas. This kind of applications will depend on resting behaviour of the target species.

Residual treatment in indoor conditions, usually restricted to interior and semi-interior wall treatments, concerning indoor environments of buildings.

Historically, the active ingredient most used for the control of mosquitoes and many other vector insects has been dichloro diphenyl trichloroethane (DDT). This active substance was endowed with a high insecticidal capacity and low acute toxicity, but at the same time had an unacceptable ecotoxicological profile. DDT has currently been banned since the 1970s in Europe and the United States of America, although it is currently still in use in many developing countries despite global production and use being undergoing modest decline since stipulation of the Stockholm Convention (Van den Berg *et al.* 2017).

The active compounds currently most in use belong to the pyrethroid family which constitutes 25% of the world market of insecticides (Soderlund *et al.* 2002). The photostable second generation pyrethroids (e.g. deltamethrin, cypermethrin, cyfluthrin) are mainly used in open environments, while the first generation non-photostable pyrethroids (e.g. allethrin, tetramethrin) are used both for repellent and killing purposes in premises (Scirocchi 1998).

Pyrethroids act by contact and ingestion, being the contact mode action facilitated by their liposolubility which provokes an easy absorption by the cuticular waxes of insects. The mechanism of action is due to the depolarisation of the neuron membrane by action on the sodium channel which is followed by the total block of nerve transmission. In the case of pyrethroids with a hydrocyanic group in the molecule, the block affects the central nervous system (CNS) and is irreversible. Toxicity towards homeothermic animals (with some exception, e.g. cats) is low. From the environmental point of view, since they are insecticides without any selectivity, the use must be carefully evaluated before carrying out a treatment in green areas (Bianco 2015).

Finally, it is necessary to remind and emphasise that pyrethroids can give rise to resistant populations (Brengues *et al.* 2003, Mint Mohamed Lemine *et al.* 2018) and it is therefore advisable, in order to avoid the onset of this phenomenon, to make a careful evaluation of the active ingredients before treatment and investigate any post-treatment failures with laboratory efficacy tests. The use of molecules belonging to other families (e.g. carbamates and organophosphorus) are no longer authorised in several continents for space treatments for toxicological and ecotoxicological reasons.

Larviciding

The larvicidal interventions are focused on the sites where larvae are developing or can potentially do it. The agronomical and mechanical strategies consist in the elimination of water resources (e.g. drainage, tanks and containers for irrigation) or in preventing that mosquitoes can reach (e.g. lids to close buckets, mosquito nets) these sites suitable for eggs oviposition. The agronomic interventions generally affect large areas with repercussions on large portions of territory, while the mechanical interventions find more point-like applications even in private areas (e.g. gardens and parks) in urban environments. Another type of mechanical fight is the application, in the mosquito breeding sites, of non-polar substances, with a specific weight lower than water and with low surface tension. These substances cause the formation of a surface layer which prevents the larvae from breathing, causing death for asphyxiation. This strategy was also applied in the past century using mineral oils, with important consequences for the environment (Harwood and James 1979). In recent years, silicone substances as well as vegetable oils have been developed to get good results but without of such negative environmental impacts (Webb and Russell 2009).

Another group of substances capable of interfering with the pre-imaginal development of mosquito larvae are the insect growth regulators (IGR's). The IGRs most commonly employed for mosquito control can be divided into Cuticle Inhibitors (CuI) and juvenile hormone analogs (JHA). The CuI are substances belonging to the benzoylureas of which diflubenzuron is the most used for the control of immature culicids. These substances act mostly by ingestion on the preimaginal larvae stages, interfering with the cuticle formation process during the pre-imaginal stages. The mechanism of action is based on the inhibition, by the active ingredient, of the enzyme chitin-synthetase. The inhibition of this enzyme prevents the deposition of N-acetylglucosamine which is essential for the formation of chitin in the cuticle and the peritrophic membrane of the intestine. Death occurs by rupture of the integument during a moulting or metamorphosis process. Diflubenzuron also has an ovicidal action and in this case acts by penetration of the chorion following contact, in particular in the newly laid eggs; the ovicidal properties are not particularly important in the fight against mosquitoes, considering that many species lay dry and not directly in water. The IC do not show selectivity in the context of invertebrates and consequently are not suitable for application in water collections communicating with protected wetlands or environments where large biodiversity of invertebrates is present.

The JHA or juvenoids are substances that only in recent decades have been deeply used in the fight against mosquitoes and other dipterans. These substances act principally by penetration the cuticle or the chorion and the mechanism of action varies according to the specific substance. The way of action is a juvenilizing effect as substances similar to neotenin or juvenoid hormone with death determined as a result of biochemical alterations in the development cycle. In the case of analogues of neotenin (e.g. Methoprene) the presence of these substances in the hemolymph prevents ecdysone from activating, in the cells of the whole organism, those chromosomal tracts in which the structure of the adult is encoded. The consequence is to keep only the larval genome active. Some other active ingredients (e.g. Pyriproxyfen) also exert an inhibitory activity of neotenine esterase, consequently preventing phenomena of metamorphosis to adult stage. The insecticidal action can be observed starting several days after application and the presence of viable larvae or pupae within the treated foci does not indicate the absence of treatment. In such cases, to verify the effectiveness of the treatment it is necessary to sample the larvae and keep them until metamorphosis into an adult when dead pupae and pre-adults could be observed. They have little or no selective action among insects even if they are endowed with low toxicity towards vertebrate animals.

Undoubtedly, larviciding through the use of microbial agents is probably the strategy that has been more quickly developed and established, especially in developed countries. The high degree of efficacy and principally the environmentally friendly approach of these biological products, which are even causing specific mortality only in culicids in some cases, are strong arguments that support the increasing interest on these biological insecticides. However, the economic cost in product manufacturing compared with other kind of insecticides, is a major concern nowadays for their introduction at large scale in developing countries with low economic resources and possibilities. More information about this topic is provided in the section of biological control.

Finally, it is necessary to consider the possibilities of chemical control. This type of applications is practically in disuse in developed countries, while it is still used in developing countries strongly affected by mosquito-borne diseases (MBD). The applications are generally done spraying water collections with pyrethroid or organophosphorus products. The action is carried out very quickly by contact and ingestion and does not show any selectivity towards both invertebrates and heterothermic vertebrates, being particularly toxic for fishes. Other chemical tool occasionally employed for mosquito larvae control is the use of metallic copper in the breeding sites. The toxic action is related to Cu ions releasing and the metal oxidation compounds. The effectiveness of metallic copper is strongly influenced by the type of metal processing (braids, wires, etc.); killing is already observable starting from concentrations of copper metal >1 g/l (Della Torre *et al.* 1993), concentrations from 4-8 to 20 g/l of metallic copper cause high mortality rates that can even reach 100% with persistence of the toxic effect for months (Della Torre *et al.* 1993, Romi *et al.* 2000). It has recently been shown how the application of Cu metallic in spray formulation with dosage of 500 µg/kg determines 100% of mortality in 2 weeks (Becker *et al.* 2015).

The use of copper metal, even in domestic and public water collections (e.g. small containers), is a practice not recommended in order to avoid excessive dispersion of heavy metals and for regulatory problems (European biocide directive) related to the active ingredient (Bellini and Veronesi 2006). It is a practice that can be evaluated urgently for outbreaks of MBD in the absence or lack of other active ingredients.

Biological control

Biological control, in the broadest sense, is defined as the reduction of the target population by the use of predators, parasites, pathogens, competitors or toxins from microorganisms (Becker *et al.* 2020, Benelli *et al.* 2016, Huang *et al.* 2017). Biological control aims to reduce the target population to an 'acceptable' level and at the same time, to avoid adverse effects to the ecosystem. As far as mosquito control is concerned, biological control measures should integrate the protection of humans from mosquitoes with conservation of the biodiversity whilst avoiding toxicological and eco-toxicological effects (Becker and Lüthy 2017, Becker *et al.* 2020, Timmermann and Becker 2017). As a result, the regulatory power of the ecosystem is maintained by protecting the existing community of mosquito predators.

The use of beneficial organisms for the control of mosquitoes was first recognised in late 19th century, when attempts were made by introducing predators such as dragonflies (Lamborn 1890). However, mass breeding and successful introduction of predators such as hydra, flatworms, predacious insects or crustaceans, often introduces a range of problems. However, such problems did not occur, or only to a limited extent, with the use of fish such as the mosquito fish, *Gambusia affinis* (Baird and Girard, 1853) (western mosquitofish) and *Gambusia holbrooki* Girard, 1859 (eastern mosquitofish), which were successfully introduced into many countries to control mosquito larvae in the early 1900s (Bellini *et al.* 1994, Chandra *et al.* 2008, Legner 1995, Walton 2007).

With the discovery and large-scale use of synthetic insecticides in the 1940s and 1950s, biological control of mosquitoes was unfortunately no longer considered to be an important method. However, the initial euphoria that greeted the success of synthetic insecticides rapidly dissipated as resistance subsequently developed within the target populations. Moreover, despite the beneficial effects of traditional insecticides, they also often have unwanted characteristics, such as their non-selectivity which frequently causes ecological damage. As public awareness of environmental issues increased, regulations controlling the application of chemicals were tightly regulated. As a result, a renaissance of the biological control of mosquitoes took precedence in the 1960s and 1970s. By 1964, Jenkins had already listed more than 1,500 parasites, pathogens and predators as potential candidates for biological control. Today, the literature on mosquito antagonists is immense (Becker *et al.* 2020, Davidson 2012, Lacey 2017, Legner 1995, Quiroz-Martínez and Rodríguez-Castro 2007).

One of the major advantages of biological control measures is that existing predators are conserved, which will then assist the control effort by preying upon newly-hatched mosquito larvae after the control operation, thereby, considerably enhancing the efficacy of the current control measures. By promoting the conservation of existing populations of predators, parasites or pathogens, there are two major strategies for the augmentation of populations of mosquito antagonists (Becker *et al.* 2020, Lacey 2017).

Inoculation refers to the release of small numbers of predators, parasites or pathogens into the habitat of the target organisms. The antagonists become established, they reproduce and multiply under favourable living conditions in the new habitat, resulting in a sustained suppression of the target population (Walton 2007).

Inundation means the release of an overwhelming number of predators, parasites, pathogens or their toxins into the mosquito habitat. Such mass release of organisms or applied pathogens (toxins) can have an immediate effect through a significant reduction of the target population. For instance, inundative control is successfully practiced with microbial pathogens which are produced in artificial cultures, e.g. *Bacillus thuringiensis israelensis* (Bti) and *Lysinibacillus sphaericus* (Lsph). Only rarely, do the antagonists become established in the habitat, for example Lsp is able to recycle under certain conditions (Becker *et al.* 1995, Lacey 2017).

A prerequisite for the successful use of predators, parasites or pathogens, is precise knowledge of the biology of the antagonist in question and its interaction with the ecosystem. For example, the introduction of foreign faunal elements as predators, risks damaging or displacing existing populations of predators. For instance, introduced fish may reduce numbers of aquatic insects, crustaceans or amphibians which would otherwise be effective predators of mosquito larvae. Rare indigenous species which do not feed on mosquito larvae may also be endangered. A thorough understanding of predator/prey or parasite/host relationships is therefore of fundamental importance for the successful and ecologically sound use of antagonists. As a result of more than 100 mill. years of evolution mosquito species are able to inhabit very different habitats, and they have developed various life strategies by adapting to habitats with very different abiotic and biotic conditions. Antagonists can only successfully reduce a target population if their own life strategy is adapted to the target population. Here some examples of the efficiency of predators, parasites and pathogens are given.

Predators

In general, predators of the immature mosquito stages are more effective than predators of the adults. As a rule, mosquito larvae and pupae are concentrated at their breeding sites and are more easily available to predators than the widely dispersed adults. Moreover, adult mosquitoes evade many predators as they are mostly nocturnal. Mosquitoes have the characteristics of typical r-strategists (meaning, a high rate of reproduction and a relatively short life cycle). Predators are particularly effective if they have a similarly high rate of reproduction and/or a high rate of feeding, like fish (Becker *et al.* 2020). Macro-organisms such as fish have been used for decades as biological control tools in many mosquito controls programs. However, fish and other predators have specific ecological requirements and can only be used where their preferred living conditions are met. The life cycle of the predator is frequently not adapted to that of the target organism so that it is unable on its own to bring about an effective reduction of the target population. Mass rearing and release of the predators or parasites is often expensive or even impossible. This limits their large-scale use in a number of specific habitats. Special attention has therefore been given to the search for microbial control agents such as Bti (Davidson 2012).

Amongst the vertebrates, fish are the most effective predators of mosquito developing stages and can be even be used in the fight against malaria (Louca *et al.* 2009). The best known fish species is the mosquito fish *G. affinis* or the guppy *Poecilia reticulata* Peters, 1859. In the United States, mosquito fish are commonly bred by mosquito abatement districts and selectively released for control in an integrated mosquito management (IMM) program. However, before a non-indiginous organisms is released its prey-selectivity and their benefit as predator in relation to their environmental damage to the existing biota has to be studied. In general, native fish should be preferred which usually don't constitute a risk to the existing biota. Feeding rates of cyprinids can exceed several hundred fourth instar mosquito larvae (Becker *et al.* 2020).

Next to fish, amphibians can be effective natural occurring predators. Here the Urodela (newts) have to be mentioned which can also consume as adults several hundred fourth instar mosquito larvae/day. In contrast to urodelans, anurans (e.g. *Rana* species) have little effect as predators. In general birds and bats are not considered to be important regulators of mosquito populations, although mosquitoes can be a relevant source of food for some species (Becker *et al.* 2020).

Invertebrate predators

Countless invertebrates are known as predators of mosquitoes especially of the larvae. The biology and importance of the predators have been investigated in numerous studies (Becker *et al.* 2020, Dida *et al.* 2015, Service 1977). Although invertebrates have been shown to be effective predators of mosquitoes, they are seldom used in control programs due to the great difficulties and the high costs involved in mass rearing of these organisms. Nevertheless, their role as consumers of mosquitoes is beyond dispute. Mosquitoes can rarely develop in large numbers at breeding sites where predacious invertebrates are abundant.

Here are only a few examples of different groups of invertebrates and their importance as predators are given: *Chlorohydra viridissima* Pallas, 1776 (Coelenterata)   10 larvae/day; flatworms (Turbellaria) *Mesostoma* sp.   5 larvae/day; spiters and mites e.g. *Argyroneta aquatica* (Clerck, 1758) >20 larvae/day; Crustacea: amoung the crustaceans the copepods are very important predators of mosquito larvae. They can consume 1-2 first instar larvae/day. They can be introduced in artificial containers for the control of the two major vectors of dengue worldwide, namely *Aedes* *aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894). Amongst the insects the nymphs of dragonflies (Odonata) are very predacious. Water bugs such as *Notonecta* spp. Linnaeus, 1758 (Heteroptera) and water beetles, such as dytiscids (Coleoptera) are very important predators and even amongst the dipterans carnivorous larvae of Culicidae and Chaoboridae are particular predators of mosquito larvae e.g. species of the genus *Toxorhynchites* Theobald, 1901 in North America or *Mochlony culiciformis* or *Chaoborus* spp. in Europe. In aquatic ecosystems rich on aquatic organisms mosquitoes can usually not proliferate in great numbers due to the positive effect of predators (Becker *et al.* 2020).

Parasites and pathogens

The most important parasites of mosquitoes are the mermithid nematodes like *Romanomermis* spp. They occur mainly in water and have been tested as biological agents in various parts of the world (Lacey 2017). Unfortunately, these parasites did not become widely used because of difficulties with transportation, maintenance of the eggs and with the sensibility of the nematodes towards particular environmental conditions which made it difficult to establish them in mosquito breeding sites (Becker *et al.* 2020).

Bacteria

The discovery of the soil bacterium *Bacillus thuringiensis* subsp. *israelensis* (Bti) in the Negev desert of Israel in 1976 and of potent strains of *Lysinibacillus sphaericus* (Lsph) in have inaugurated a new chapter in the control of mosquitoes and blackflies (Becker and Margalit 1993, Becker *et al.* 2020, Mulla *et al.* 1990). The new subspecies of *B. thuringiensis* is highly toxic to larvae of most mosquito species and to blackfly larvae and to less extent to some members of other nematoceran families. New strains of Lsph, such as strain 2362 isolated from an adult blackfly in Nigeria (Weiser 1984) are much more potent than the first isolates and are particularly active against larvae of *Culex* species and *Anopheles gambiae*, the major malaria vector in Africa.

The discovery of these microbial control agents marked the breakthrough in biological control, because of the special abilities of these microbial agents. Their protein crystals are highly toxic to target organisms and extremely environmentally safe. Mass production of the bacteria, the availability of efficient formulations and the easy handling of the formulated products make microbial control tools a successful new weapon against nuisance and vector mosquitoes.

Biological control in the context of Nature Reserves, Zoos and other areas with protected wildlife

Dispose of tools and strategies to reduce mosquito populations without having impact on the rest of biocenosis is particularly important in protected territories where wildlife should be conserved. Protected wetlands are good example of these environments where mosquito control strategies must be carefully analysed in terms of cost-benefit before to be applied. The basis of these IMMP in wetlands should be related to the employment of non-chemical larvicides (Martinou *et al.* 2020), together with the habitats management for mosquito source reduction, as well as the introduction of natural predators (always local species without collateral impact on the rest of the fauna). This is particularly interesting since wetland creation, conservation or restoration projects often ignore possible impacts posed by mosquito population dynamics, mosquito-borne pathogens, nor do they always include mosquito management plans (Willott, 2004).

According to different institutions involved in Mosquito Management on Wildlife Refuges (US Fish and Wildlife Service, 2018), larvicides are generally preferred over mosquito adulticides in protected areas for several reasons:

a. Prevention: larviciding means the real prevention since the use of mosquito larvicides prevents the appearance of the blood feeding adults, which represent the nuisance stage of the cycle.
b. Residuality: mosquito larvicides can provide up to a month of control (3-5 weeks), rather than the few hours or days provided by fogging with adulticides.
c. Ecotoxicology: the commonly used mosquito larvicides are less toxic than the adulticides and are applied in such a way that there is much less non-target fauna exposure.
d. Cost-benefit balance: mosquito larvicides generally are applied to smaller areas than are adulticides.

Other key areas or territories where IMMP could have a crucial role in animal health protection are urban zoos (Quintavalle Pastorino *et al.*, 2015). Cases of mosquito-borne diseases like avian malaria (Grim *et al.,* 2004; Martínez-de la Puente *et al.,* 2020), Eastern Equine Encephalitis Virus (Tuttle *et al.*, 2005), West Nile Virus (Jett & Ventre, 2012), Usutu Virus (Weissenböck *et al.*, 2002) and Dirofilariasis (Sano *et al.*, 2005) have been documented in zoos. In these zoological facilities, once again the selective employment of biolarvicides and proper management of stagnant waters in order to reduce mosquito larval biotopes are essential environmental actions from the point of view of animal health.

Biotechnology and innovative tools for mosquito control

The need to explore new complementary and innovative mosquito control strategies and tools, beyond the traditional ones (insecticides for both adulticiding and larviciding interventions, and breeding sites removal through 'door to door' programs and citizen awareness projects) is widely recognised since relevant nuisances and/or mosquito-borne diseases are still present in the vast majority of countries from all over the world. This search for new alternatives in mosquito control is particularly urgent in urban environments where current control measures are highly ineffective in many countries due to the large availability of cryptic mosquito larval breeding sites, most of them in private areas where mosquito control interventions are difficult to implement by public agencies. Moreover, the increasing evidence in relation to chemical insecticides resistance phenomena in mosquitoes emphasises even more the need to evaluate alternatives for effective and efficient mosquito control programs. In recent years a lot of effort has been done in order to develop strategies like genetically modified mosquitoes (GMO), sterile insect technique (SIT) through mosquito irradiation, mosquito populations suppression employing the incompatible insect technique (IIT) by means of *Wolbachia* manipulation and introduction, or effective attractive toxic sugar baits (ATSB), among others. All these approaches aim to complement traditional vector control plans mostly based on chemical or biological insecticides applications with the last goal to implement integrative mosquito management Programs (IMMP).

Genetically modified mosquitoes

As occur with other non-GM methods, such as *Wolbachia*, there two major theoretical types of effects in nature that we can achieve when GMM are released in the field. First one is called 'population suppression' and aims to reduce or suppress mosquito population in order to minimise the impact of the species either simply in terms of nuisances due to mosquito bites or in relation to pathogens transmission. GMM can achieve this in several ways, like provoking biasing against the development of female progeny (sex-ratio distortion), reducing female fertility, introducing a mechanism that incapacitates or kills young female mosquitoes, and even some methods to shorten significantly the lifespan of female mosquitoes (WHO 2004). The second one is named 'population replacement' and has the focus on vector competence shortening by means of reduce the inherent ability of individual mosquitoes to transmit a given pathogen. In case of GMM the introduction of engineered DNA and/or the manipulation of endogenous genes inhibit pathogen replication within the mosquitoes, making them refractory to transmission (WHO 2004). Accurate releases of these GMM try to provoke changes in local wild populations, 'replacing' their inherent ability to spread the targeted pathogen with a reduced or eliminated transmission capability.

Several studies and field releases of *Ae. aegypti* and *An. gambiae* GMM, major vectors of dengue and malaria, have been conducted in recent years in several territories of Asia, America and Africa (Beisel and Ganle 2019, Carvalho *et al.* 2015, Subramaniam *et al.* 2012). Some of them showpromising results in terms of mosquito populations reduction (Carvalho *et al.* 2015, Gorman *et al.* 2016). However, there is a strong debate in the scientific and ethical community about likely benefits and risks that this technique could have for individuals (Macer 2005, Resnik 2017), communities and the environment, mostly due to unknown medium and long-term impacts on ecosystems.

Sterile insect technique

The SIT is based on the continuous release of large amounts of sterilised mosquito males, traditionally by means of irradiation, with the last goal to suppress vector mosquito populations. As occur with other control strategies focused on massive mosquito releases, like IIT or GMM, major challenges with SIT are related with the achievement of optimal operational costs in relation to facilities, human resources and technology need for massive mosquito rearing. Furthermore, other parameters that require an accurate scientific development like sex separation systems, adequate release methodology and exhaustive field entomological evaluations of variables like survival and sexual competition of released males, definition of suitable moments for releases according to populations phenology and monitoring the efficacy of actions conducted, among others.

Around 10 small scale field trials are projected to be held in America, Asia, Africa and Europe in next years (Bouyer *et al.* 2020), having by now some interesting preliminary results with *Ae. aegypti* and *Ae. albopictus* (Bellini *et al.* 2013, Kittayapong *et al.* 2019), both major vectors of urban arboviruses worldwide.

Wolbachia

Wolbachia is an endosymbiotic bacteria which is estimated to be naturally present in around 66% of all insect species, showing a wide range of ecological interactions, varying from parasitism, commensalism and mutualism, with their eukaryotic host cells (Dobson *et al.* 2002, Jeyaprakash and Hoy 2000). Different *Wolbachia* strains can generate parthenogenesis, feminisation and cytoplasmic incompatibility (CI) on their hosts (Jeyaprakash and Hoy 2000, Werren *et al.* 2008), being consequently an interesting biotechnological tool for insect population control. As has been described previously for GMM, there are two main approaches in the employment of *Wolbachia* to interfere on wild mosquito populations: mosquito suppression and mosquito replacement (Inácio da Silva *et al.* 2021). The first one is known as IIT and occurs when males reared in laboratory conditions which are previously infected with specific Wolbachia strains, are later released in the field to mate and reproduce with wild *Wolbachia*-free females, which finally leads to CI between gametes and the subsequent absence of viable offspring (Nazni *et al.* 2019, Yen and Barr 1971). Regarding to the second one here the goal is to replace wild populations by releasing both males and females which are infected with a specific strain of *Wolbachia* that can reduce the arbovirus replication in the target mosquito (Aliota *et al.* 2016, Dutra *et al.* 2016), so basically there is a substitution of natural populations of mosquitoes by artificial ones in order to finally reduce the vector competence of local populations. This is done thanks to the vertical transmission of *Wolbachia* from females to offspring.

Autodissemination of insecticides

One of the critical issues in the control of *Aedes* mosquitoes which usually breed in small containers of urban habitats, is the successful neutralisation of cryptic/inaccessible breeding sites in private areas. This key operational problem to control inaccessible immature mosquito habitats has been proposed in several studies to be partially solved with the employment of autodissemination strategies (Unlu *et al.* 2020). Autodissemination uses adult mosquitoes as a vehicle to treat containers that are inaccessible to direct treatments. This adult contamination can occur through direct contact of mosquitoes with treated materials (Tsunoda *et al.* 2013) or dissemination stations, such as modified ovitraps (Devine *et al.* 2009). Once adults are contaminated with the control agent, both males and females can disperse the insecticide; females usually contaminating new aquatic cryptic habitats due to oviposition actions (vertical transfer) and males also contaminating alternative females during mating thanks to polygamic behaviour (horizontal transfer). The control agents usually employed correspond to insect growth regulators (IGR's), being usually pyriproxyfen the most common insecticide. Several field studies focused on *Ae. aegypti* and *Ae. albopictus* showed promising results in terms of populations reduction (Caputo *et al.* 2012, Devine *et al.* 2009). However, there are some factors that strongly affect the degree of efficacy of autodissemination strategies which should be previously considered in each working area, such as the adult mosquito abundance, the distance between aquatic breeding sites and dissemination stations, and urban structure and topography (Seixas *et al.* 2019). Beyond *Aedes* mosquitoes and pyriproxyfen, recent researchers provided interesting results of autodissemination control assays with *Anopheles* mosquitoes and other IGR's like novaluron or triflumuron (Swale *et al.* 2018).

Attractive toxic sugar baits

This strategy is based on the sugar feeding behaviour that both males and females of mosquitoes exhibit, attracting them to bait solutions usually composed by sugar, an attractant, and an oral toxin with insecticides properties. The management of insecticide baits in case of other pest insects (cockroaches, ants, termites or non-biting flies) is conventionally widely use today. However, the development of effective and strongly attractive insecticide baits in case of hematophagic insects remains a challenge nowadays. This is one of the reasons why since the first studies conducted more than 50 years ago with promising results (Lea 1965), unfortunately this tool has never been developed and implemented in vector control programs at large scale. In this period of time, several researches have obtained interesting results in terms of mosquito control with *Aedes*, *Anopheles* and *Culex* mosquitoes, employing different substances as insecticides like boric acid, neonicotinoids, and fipronil among others (Müller and Schlein 2008, Müller *et al.* 2010a,b, Naranjo *et al.* 2013, Revay *et al.* 2014). ATSB solutions can be directly applied on vegetation or alternatively in bait stations that attract mosquitoes from a large area (the technique of lethal baits is usually named 'attract and kill'). This second type of application is suspected to have lower impact of non-target sugar feeding insects of the environment. Precisely this low knowledge of ATSB potential impact on non-target organisms, is another drawback of this strategy (Khallaayoune *et al.* 2013).

The degree of alternative sugar feeding natural sources in the control area is also another key issue to evaluate the efficacy of ATSB.

Mass trapping

Mass trapping is based on the installation of enough number of effective mosquito traps to provoke substantial reductions on mosquito populations during long terms in a specific intervention area. Depending on the target species, landscape/habitat structure (urban/rural areas, wetlands, forests, etc.) and degree of implementation of other vector control activities inside the IMMP (larviciding, adulticiding, breeding sites removal, etc.), different types of traps can be selected to achieve optimal results of mass trapping. At the end of last century, traditional ovitraps (consisting of a small black plastic container filled with water and a wooden sampling paddle to allow egg laying of mosquito females) have been modified to be lethal for ovipositing females by impregnating the oviposition substrate with insecticides (Zeichner and Perich 1999). These traps, called 'lethal ovitraps (LO)', have been deeply studied to reduce *Aedes* mosquitoes in urban areas showing positive results in trials based on massive utilisation of these devices (Ocampo *et al.* 2009, Perich *et al.* 2003, Sithiprasasna *et al.* 2003). Another modification of traditional ovitraps has derived in a tool called 'sticky ovitrap (SO)', which contains a sticky surface which also catch gravid females and resting males (Ritchie *et al.* 2003). Moreover, the last design modification of these ovitraps has allowed the development of 'autocidal ovitraps (AO)', which allow females oviposition (that is even enhanced thanks to specific attractants) but inhibit adult hatching and also allow gravid females catching (Barrera *et al.* 2014, Mackay *et al.* 2013). One of the biggest problems in the effective implementation of mass trapping strategies with ovitraps, is the negative effect of alternative and cryptic oviposition sites which are competing with LO, SO or AO. Consequently, there is a need for community engagement and participation in order to reduce alternative water-holding containers and maximise the lethal effects of ovitraps on local mosquito populations (Johnson *et al.* 2017). Beyond ovitraps, other types of conventional adult mosquito traps have been used in different studies to evaluate their significative effect on populations reduction. Various adult traps models using different attractants like ultraviolet/green-white light, carbon dioxide (CO_2), visual cues, animal baits or synthetic lures are commonly employed in the IMMP. Centers for Disease Control and Prevention (CDC) light traps, the Encephalitis-Virus-Surveillance (EVS) traps or Mosquito Magnet traps are some of these devices frequently used, but most robust scientific data about the potential use for mass trapping correspond to BG-Sentinel (BGS) traps, especially in urban areas (Akhoundi *et al.* 2018, Degener *et al.* 2014, Englbrecht *et al.* 2015, Lühken *et al.* 2014).

Complementary tools and strategies to enhance IMMP

In the current context of global change, where globalisation and climate change create new risk scenarios for the proliferation of certain vectors, the introduction of new exotic and invasive species and the consequent risk of disease transmission, it has become necessary to incorporate new technologies and integrate them into surveillance and control strategies. This allows to tackle mosquito problems from a multidisciplinary perspective, incorporating different elements that have helped to manage the problems more efficiently. In this sense, the use of geographical information systems applied to a physical and management context or the implementation of educational workshops as a source of information and awareness are clear examples of these improvements.

Geographic information systems

Geographic information systems (GIS) are nowadays widely used by professionals in mosquito research and control for computing spatially related data. There is no doubt that GIS has revolutionised the mosquito vector-management, becoming an essential tool for its monitoring and control (Bonnefoy *et al.*, 2008). Modern information technology allows the integration of GIS systems with database technology, and with digital mobile field data collection systems supported by a global positioning system (GPS). The ability to link information provides the user with a better understanding of spatial phenomena and their relationships that may not be apparent without such advanced techniques. The application of GIS allows precisely mapping locations of certain features important to the control strategy, mapping quantities and densities, e.g. over period of time in order to forecast future conditions (Becker *et al.* 2020, Khormi and Kumar 2015). Thus, GIS and information technology can greatly improve survey, logistics and documentation of mosquito control operations. The possible applications range from direct digital site-mapping using GPS assisted mobile devices to timely aggregation of operational reports. A spatially referenced database containing all features of interest is the basis for all data collection and analysis and allows for example applications as follows: Spatial analysis to determine relationships between human nuisance or disease and breeding-sites (calculation of buffer-zones, map- and database query); forecasting of time and location of appropriate control activities, based on correlations between the spatial occurrence of triggering events for larval development (e.g. water levels and flooding areas, local weather data, the potential of larval development sites, and the results of current survey data); preparation of operational maps to improve logistics, calculate the quantities of control materials and manpower required, and to calculate the duration and cost of treatment; storage of historical-site profiles and related attribute data on the basis of operational maps, enables future potential larval development, resulting from dynamic triggering events, to be predicted; GPS-assisted operations allow the tracking and direct digital documentation of field activities (e.g. aerial application). Employing a user-defined database allows precise reports and documentation of survey and control activities. The results can be visualised and printed in the form of standardised thematic maps, graphics or tables. Nowadays, knowledge of the territory and the factors favouring mosquitoes has made it possible, through spatial and geographical analysis of georeferenced data, to draw up predictive models of the risk of mosquito proliferation in a vector surveillance framework (Hay *et al.* 2006, Parra-Henao 2010). In addition, the system allows information to be added to each point on different parameters, whether structural, biological or ecological, in order to later spatially analyse this data, interpret the information in the territory and propose targeted actions which would otherwise be difficult to decide upon (Barker *et al.* 2017). Thus, identifying and register biological, social, economic, geographic and environmental data will be of great help in the development of risk maps, or predictive models that can have different uses, from the surveillance of invasive alien species and their possible routes of introduction, the selection of insecticides or the reduction of risks derived from vector-borne diseases, such as Dengue, Zika, West Nile virus or Chikungunya (Kiltron 1998).

Citizen and management science

These two elements are important in the development of IMMP in different cities of the world; the use of citizen science for the improvement municipal surveillance and control procedures, and the implementation of educative projects which collaborate with training, awareness and sensitisation through the implementation of educational workshops on vectors and their management for schoolchildren.

One example that illustrates the incorporation of citizen science is the MosquitoAlert platform, a science project to research, monitor and control the spread of disease-transmitting mosquitoes. Through the appMosquito Alert, citizens can help scientists detecting adult mosquitoes and their breeding sites (sewers, fountains, containers...) by sending pictures which are geolocated. This fact has allowed different administrations to incorporate this information into their IMMP. Moreover, recent research has revealed that this citizen science project provides a reliable and scalable tool to track disease-carrying mosquitoes (Palmer *et al.* 2017). Other citizen science project launched in 2012 named 'Mückenatlas' (mosquito atlas) show similar results in terms of its usefulness for the mosquito surveillance and control programmes (Pernat *et al.* 2021).

Moreover, it is well known that local communities are a key actor to reduce mosquito populations, since several studies have revealed that active education can lead to significant reduction in peridomestic container mosquito habitats (Bodner *et al*. 2016, Healy *et al.* 2014).

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