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REVIEW ARTICLE

Contributions of *Anopheles* larval control to malaria suppression in tropical Africa: review of achievements and potential

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Abstract. Malaria vector control targeting the larval stages of mosquitoes was applied successfully against many species of *Anopheles* (Diptera: Culicidae) in malarious countries until the mid-20th Century. Since the introduction of DDT in the 1940s and the associated development of indoor residual spraying (IRS), which usually has a more powerful impact than larval control on vectorial capacity, the focus of malaria prevention programmes has shifted to the control of adult vectors. In the Afrotropical Region, where malaria is transmitted mainly by *Anopheles funestus* Giles and members of the *Anopheles gambiae* Giles complex, gaps in information on larval ecology and the ability of *An. gambiae sensu lato* to exploit a wide variety of larval habitats have discouraged efforts to develop and implement larval control strategies. Opportunities to complement adulticiding with other components of integrated vector management, along with concerns about insecticide resistance, environmental impacts, rising costs of IRS and logistical constraints, have stimulated renewed interest in larval control of malaria vectors. Techniques include environmental management, involving the temporary or permanent removal of anopheline larval habitats, as well as larviciding with chemical or biological agents. This present review covers large-scale trials of anopheline larval control methods, focusing on field studies in Africa conducted within the past 15 years. Although such studies are limited in number and scope, their results suggest that targeting larvae, particularly in human-made habitats, can significantly reduce malaria transmission in appropriate settings. These approaches are especially suitable for urban areas, where larval habitats are limited, particularly when applied in conjunction with IRS and other adulticidal measures, such as the use of insecticide treated bednets.

Key words. *Anopheles funestus*, *Anopheles gambiae* complex, bacterial larvicides, drainage, environmental management, irrigation, IRS, ITN, IVM, malaria vector control, mosquito larvae, larval control, larvicide, larvivorous fish, water management, tropical Africa.

Introduction

Larval control of malaria vector *Anopheles* mosquitoes is a well-proven preventive method that has become neglected, but deserves renewed consideration for malaria control progammes in the 21st Century. Prior to the 1940s, antimalaria operations generally focused on control of the larvae of vector species (W.H.O., 1982; Kitron & Spielman, 1989; Litsios, 1996). With the discovery of the insecticidal properties of dichlorodiphenyltrichloroethane (DDT), the primary vector control method became indoor residual insecticide spraying (IRS) targeting adult mosquitoes, although supplemental chemical larviciding also occurred in some areas during the malaria eradication campaigns of the 1950s and 1960s (Pampana, 1969). After the goal of malaria eradication was abandoned in 1969, international support for centrally organized malaria control programmes

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based on indoor residual spraying, case detection and treatment declined (Oaks *et al.*, 1991; Bradley, 1998). Increasing resistance to antimalarial drugs and insecticides, inadequate health care systems, population displacement and declining community acceptance have further reduced the effectiveness of the malaria control approaches developed in the eradication era (W. H.O., 2000). At the same time, ecological changes driven by deforestation, human migration and unmanaged urbanization have increased the densities of human hosts and vector breeding sites in some malarious regions (Gratz, 1999; Robert *et al.*, 2003). Thus, malaria remains a serious and growing health problem in many developing countries, particularly in the Afrotropical region (sub-Saharan Africa), which was largely ignored during earlier eradication efforts. Given the variability of the disease pathogens, the vectors and the vulnerability of particular human populations, the World Health Organization (W. H.O.) stresses the need for a range of malaria control approaches, including selective and sustainable vector control, in their Global Malaria Control Strategy (W.H.O., 1993). Recently, W.H.O. has issued a Global Framework for Integrated Vector Management (IVM), stressing the importance of evidence-based combinations of vector control methods (W.H.O., 2004). Vector control interventions such as house spraying or insecticide treated nets are often highly effective but are also vulnerable to the development of resistance and vector avoidance behaviours (Killeen *et al.*, 2002a). Although unlikely to replace insecticide-based adult mosquito control, improved chemical and non-chemical larval control methods could offer sustainable supplements to other malaria vector and pathogen control efforts (Killeen *et al.*, 2000). In this review, we explore the possible role of larval control in malaria prevention, particularly in sub-Saharan Africa, by reviewing the existing literature on anopheline larval control methods, focusing on large-scale field studies that have tested the impacts of these methods on vector density and, where possible, on malaria transmission and incidence.

Malaria is a complex disease caused by four different pathogens and vectored by approximately 60 species of anopheline mosquitoes (Oaks *et al.*, 1991). Many successful malaria vector control programmes have focused on the adult female mosquito population because shortening the length of this life stage has a major impact on its vectorial capacity and hence reduces malaria transmission (Macdonald, 1957). Furthermore, rather than vector control operations having to seek out (larval) vectors, adult females make themselves vulnerable to control interventions when they seek human hosts. When used correctly in suitable transmission conditions, both IRS and insecticide treated bednets (ITNs) can dramatically reduce the burden of malaria by killing adult female mosquitoes when they come to take human bloodmeals (Curtis *et al.*, 1990; Alonso *et al.*, 1991; Coosemans & Carnevale, 1995; W.H.O., 1995; Lengeler & Sharp, 2003). Although the primary goal of such interventions is the reduction of human/vector contact rather than reduction of the vector population, these control methods may also suppress the local vector population under certain circumstances (Magesa *et al.*, 1991; Robert & Carnevale, 1991; Gimnig *et al.*, 2003).

By contrast, larval control measures are intended to reduce malaria transmission indirectly by reducing the vector population density near human habitations. As the larvae are exclusively aquatic, their distribution is determined by the locations of suitable water bodies. Immature stages prefer slow-moving or still water in which they can stay close to the surface with their breathing orifices open to the air. Unlike some other mosquito genera, anophelines require relatively clean water for development (Service & Townson, 2002). Before a larval control intervention can be implemented, the majority of the vector larvae's productive breeding sites must be identified. If the number of breeding sites is extremely large or many sites are inaccessible or ephemeral, larval control may not be feasible. Larval control appears promising in urban areas, given the high density of humans relative to the number of breeding sites (Keiser *et al.*, 2004). Even in rural areas, recent evidence from an ITN study site in Western Kenya suggests that once adult populations have been reduced through high ITN coverage, the larval population is also reduced (W. Hawley, personal communication). Furthermore, the breeding sites producing late instars and pupae in this setting appear to be fewer and aggregated, suggesting that larval control measures could be especially effective where there is a high level of ITN coverage. A model by Killeen *et al.* (2000) of the combined impacts of ITNs and larval control predicted that a 50% reduction in vector emergence from breeding sites could contribute to an overall 15–25-fold reduction in entomological inoculation rate (EIR), even in highly endemic areas.

One advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and, unlike adult mosquitoes, cannot easily avoid control measures (Killeen *et al.*, 2002a). Larval control may be particularly valuable in regions where the primary malaria vector is exophilic and/or bites before people are in bed, making indoor residual spraying and impregnated bednets less effective, as in parts of Eritrea (Shililu *et al.*, 2004). Even in areas where temporary wet season breeding sites are too numerous to control, management of the more limited number of dry season habitats might be feasible (Fillinger *et al.*, 2004).

Larval ecology of major Afrotropical malaria vector species

The development and implementation of effective larval control methods requires adequate information about the distribution and behaviour of vector larvae (Rozendaal, 1997). Table 1 lists some of the most important vector species and their typical larval habitats. Certain environmental conditions are particularly important in determining larval habitat suitability for the different anopheline vectors, including size and permanence of the water body, water salinity and turbidity, amount of sunlight, and presence of emergent or floating vegetation (Service, 1989). One complication of larval control is the variability in larval habitat requirements between vector species and even within different populations of the same species. Therefore, larval control practices targeting one vector in a region may be inappropriate for another vector species [e.g. *Anopheles maculatus* Theobald and *Anopheles umbrosus* (Theobald) in Malaysia; Konradsen *et al.*, 2004].

Malaria prevention using larval control is not currently practiced in most of sub-Saharan Africa, although programmes to

Table 1. Major malaria vectors and their breeding habitats.

Region of activity	Vector species	Main larval habitats	References
Afrotropical Region: sub-Saharan Africa	*Anopheles gambiae* Giles s.*l*., mainly *Anopheles arabiensis* Patton and *Anopheles gambiae* Giles s.*s*.	Temporary sunlit pools without vegetation; wells and ricefields	Gillies & de Meillon (1968); Githeko *et al.* (1996); Robert *et al.* (1998); Gimnig *et al.* (2001)
	Anopheles funestus Giles	Large water bodies with emergent vegetation	Gillies & de Meillon (1968); Gimnig *et al.* (2001)
Neotropical Region: central and South America	*Anopheles albimanus* Wiedemann	Marshes or pools with emergent vegetation or algae	Service & Townson (2002)
	Anopheles pseudopunctipennis Theobald	Edges of rivers and streams	Zimmerman (1992)
	Anopheles darlingi Root	Edges of rivers: small, shady pools with emergent vegetation	Manguin *et al.* (1996)
	Anopheles culicifacies Giles s.*l*.	Human-made pits, ricefields, pools at edges of streams,	Konradsen *et al.* (1998); Yapabandara *et al.* (2001)
South-east Asia	*Anopheles minimus* Theobald	Edges of streams and seepages; ricefields	Service & Townson (2002)
	Anopheles stephensi Liston	Human-made containers; small, sunny pools	Sharma (1996)
Western Pacific	*Anopheles sundaicus* (Rodenwaldt) *s.l.*	Brackish water in coastal lagoons	Takken *et al.* (1990); Service & Townson (2002)
	Anopheles maculates Theobald s.*l*.	Sunny edges of hillside streams	Konradsen *et al.* (2004)

eliminate larval habitats existed in certain economically important areas in the early 20th Century (Killeen *et al.*, 2002a). It has been suggested that the shift in focus to the adult vector since the DDT era is itself an obstacle to the adoption of larval controls (Stevens, 1984; PEEM, 1986; Killeen *et al.*, 2002a). In addition, the lack of information and complicated vector biology of the three main vector species, *Anopheles gambiae* Giles *s.s.*, *Anopheles arabiensis* Patton (referred to collectively as *An. gambiae sensu lato*) and *Anopheles funestus* Giles, inhibit implementation of successful larval-based malaria control (Gimnig *et al.*, 2001). Some species may be found in a wide range of habitats, making identification and treatment of all key breeding sites difficult. For example, larvae of the sibling species *An. gambiae s.s* and *An. arabiensis* may be found in rice fields, borrow pits, temporary pools, drinking water vessels and even the water collecting in a cow's hoofprint (Service & Townson, 2002). Characterization of larval habitats is further complicated in Africa because *An. gambiae s.l.* is a species complex which, in addition to the two most important vector species, *An. gambiae s.s* and *An. arabiensis*, includes minor vectors such as *Anopheles merus* Dönitz and *Anopheles melas* Theobald and non-vector species such as *Anopheles quadriannulatus* A and B. Larvae of these different species may colonize different breeding habitats, but cannot be distinguished using morphological features. In spite of these difficulties, however, successful malaria prevention programmes targeting *An. gambiae s.l.* and *An. funestus* larval habitats were implemented in several mining towns in Zambia in the 1930s and 1940s (Utzinger *et al.*, 2001).

The development of polymerase chain reaction methods to distinguish species within the *An. gambiae* complex (Charlwood & Edoh, 1996) has allowed more precise study of the larval ecology of *An. gambiae s.s* and *An. arabiensis* during the past decade. Several studies observed temporal variation in abundance of the two species, suggesting that *An. arabiensis* is better adapted to dry, hot conditions (Githeko *et al.*, 1996; Gimnig *et al.*, 2001; Koenraadt *et al.*, 2004) whereas *An. gambiae s.s*. is a better competitor than *An. arabiensis* under ideal breeding conditions (Schneider *et al.*, 2000). However, researchers have been unable to identify consistent breeding habitat preferences of either species (Charlwood & Edoh, 1996, Tanzania; Minakawa *et al.*, 1999, western Kenya; Gimnig *et al.*, 2001, western Kenya; Edillo *et al.*, 2002, Mali; Minakawa *et al.*, 2002, western Kenya), although larvae of both species are less common in deep water with floating or tall emergent vegetation (Gimnig *et al.*, 2001; Minakawa *et al.*, 2004). Both species prefer sunny, temporary puddles or pools, but are 'likely to breed in any habitat that happens to be available' (Holstein, 1954; quoted in Fillinger *et al.*, 2004). *Anopheles gambiae s.l.* also appears to adapt quickly to new habitat sites, such as trash-filled pools (sometimes containing raw sewage) in urban areas (Chinery, 1984; Keating *et al.*, 2003). In a 20-month continuous study of *An. gambiae s.l.* breeding habitats in western Kenya, Fillinger *et al.* (2004) observed that virtually all water bodies, regardless of permanence, could be productive breeding sites periodically.

Although *An. arabiensis* and *An. gambiae s.s.* larvae are found in many types of breeding habitats, those created by human activity may be particularly important in terms of malaria transmission. Building construction frequently creates structures that collect rainwater and provide excellent breeding sites near human dwellings (Carlson *et al.*, 2004; Fillinger *et al.*, 2004). In the highlands of western Kenya, Carlson *et al.* (2004) found significantly more anopheles larvae and fewer predators in pits left from brick-making than in a nearby natural swamp. Fillinger *et al.* (2004) found that 70% of available breeding sites

were human-made and included many cement-lined pits left from brick house construction. Agricultural practices may also create new breeding sites or increase the productivity of certain breeding sites. Irrigated rice fields are known to breed *An. gambiae s.l.*, particularly early in the season before the rice vegetation canopy is well-developed (Lacey & Lacey, 1990; Ijumba & Lindsay, 2001; Klinkenberg *et al.*, 2003). Other irrigation structures, such as wells, may provide permanent breeding sites with few larval predators close to human habitations, as Robert *et al.* (1998) observed in urban Dakar, Senegal. Breeding sites created by the construction of thousands of small dams in Ethiopia have been shown to increase the incidence of malaria in communities near the dams by a factor of seven (Ghebreyesus *et al.*, 1999). In central Ethiopia, Ye-Ebiyo *et al.* (2000; 2003) found that the presence of maize pollen from surrounding gardens enhanced the development of *An. gambiae s.l.* larvae even under unfavourable conditions of crowding and high water turbidity. Papyrus swamps cleared and drained for crop-growing in south-west Uganda increased minimum and maximum temperatures significantly, as well as increasing malariometric indicators, suggesting an increased risk of malaria transmission in highlands previously at low risk (Lindblade *et al.*, 2000).

The colonization of human-made breeding habitats is particularly important for malaria transmission in the growing urban centres of Africa where an estimated 200 million Africans live today, approximately 25% of the total population (Keiser *et al.*, 2004). Although entomological inoculation rates (the number of infective mosquito bites a person receives per year) are significantly lower in urban centres than rural areas, they are significant (average of 45.8 annually) in the extensive peri-urban settlements associated with cities (Robert *et al.*, 2003). Although water pollution and lack of suitable natural habitats probably limits the number of breeding sites in city centres, haphazard building and water management, as well as small-scale agriculture typical of peri-urban areas, can provide extensive larval habitat for *An. gambiae s.s* and *An. arabiensis* (Khaemba *et al.*, 1994; Robert *et al.*, 1998; Keating *et al.*, 2003) and occasionally *An. funestus* (Lindsay *et al.*, 2004). Keating *et al.* (2003) found a positive association between human density and larval breeding sites up to a point in two Kenyan towns, Kisumu and Malindi. Most breeding sites were human-made and frequently were associated with water delivery or storage. In a recently developed suburban area in the Kenyan highlands, Khaemba *et al.* (1994) found temporary pools created through construction activities to be the main breeding sites during the rainy season, whereas permanent dams and, to a lesser extent, natural swamps were important during the dry season. Brick pits and tyre-ruts were important breeding sites in a peri-urban neighbourhood of Kampala, Uganda (Lindsay *et al.*, 2004). Under some conditions, identification and treatment of key vector breeding sites created by human activity in urban and peri-urban areas may be feasible and cost-effective, particularly in the dry season.

By contrast to *An. gambiae s.l.*, larvae of the third major African vector, *An. funestus*, typically inhabit large, permanent water bodies with aquatic vegetation, generally preferring shaded rather than sunny breeding sites (Gillies & de Meillon, 1968; Gimnig *et al.*, 2001; Minakawa *et al.*, 2002). Mature rice fields provide highly productive breeding habitat for *An. funestus* in East Africa and Madagascar, but not in West Africa (Marrama *et al.*, 1995). *Anopheles funestus* is generally not associated with urban malaria transmission, although as mentioned previously, it was found in small numbers in Kampala (Lindsay *et al.*, 2004). At low population densities, *An. funestus* larvae are difficult to find because they hide below the water surface for extended periods of time (Gillies & de Meillon, 1968; Marrama *et al.*, 1995), a behaviour that can complicate efforts to identify positive vector breeding sites. The larger, permanent and more obvious nature of the preferred *An. funestus* breeding habitats, however, may facilitate larval control efforts, particularly if the control strategy targets *likely* (as opposed to *proven*) breeding sites.

Although additional studies are needed, the existing literature on the larval ecology of the principal Afrotropical malaria vectors suggests that larval control targeting human-made breeding sites could have significant impacts on vector populations under certain transmission conditions (Carlson *et al.*, 2004). Dry-season management or treatment of permanent water bodies could also play a valuable role (Mutuku *et al.*, 2006). A malaria transmission model developed by Killeen *et al.* (2000) suggests that larval control could also play a supplementary role in reducing malaria EIR even in highly endemic areas. Below, we review the published literature on large-scale operational trials of different types of larval control techniques, both from Africa and other parts of the world.

Environmental management of malaria vectors

Since the discovery of the role of *Anopheles* mosquitoes in malaria transmission over 100 years ago, malaria control programmes targeting potential mosquito larval breeding sites have helped reduce or eliminate malaria transmission. Habitat elimination or modification efforts include general programmes to reduce the abundance of all mosquitoes as well as more targeted species sanitation projects directed at the principal malaria vectors. The concept of modifying vector habitat to discourage larval development and/or human vector contact is generally referred to as environmental management or source reduction (Singer *et al.*, 2005). The W.H.O. (1980) defined environmental management as:

> 'The planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with man with a view to preventing or minimizing vector propagation and reducing man-vector-pathogen contact. This approach, which should be carried out prudently and skilfully, is naturalistic and involves an attempt to extend and intensify natural factors which limit vector breeding, survival and contact with man.'

The specific techniques of environmental management are generally grouped into three main categories – environmental modification, environmental manipulation, and modification of human habitations/behaviours (W.H.O., 1982; Ault, 1994). The first two categories generally target the larval stages, whereas the third may also target adult vectors. This review will focus on

efforts to reduce or eliminate larval breeding habitats. A number of manuals and reviews explain specific environmental management techniques in detail (W.H.O., 1982; Rafatjah, 1988; Rozendaal, 1997; FCCMC, 1998). These sources include extensive technical information on major engineering projects such as large-scale dams. Environmental management has proven valuable in preventing or mitigating malaria and other vector-borne diseases sometimes exacerbated by large-scale water projects (Lim *et al.*, 1987; Hunter *et al.*, 1993).

Permanent environmental modification of mosquito larval habitat

Environmental modification involves a physical change (often long-term) to potential mosquito breeding areas designed to prevent, eliminate, or reduce vector habitat. The principal methods of achieving these changes include drainage, land leveling, and filling (W.H.O., 1982). Draining operations include creating ditches or drains to keep water moving and to carry water used as breeding sites by mosquitoes away in a managed way. Drains may be lined or unlined and located at the surface or subsoil level. In some instances, marshes have been drained through pumping (Takken *et al.*, 1990).

In addition to complete elimination of wetlands, modification projects can involve creating channels to increase water flow in areas of standing water, filling small ponds or water-collecting depressions, or changing banks of water impoundments to reduce mosquito populations. Because slow-moving pools with heavy vegetation in rivers and streams can create larval breeding sites for certain vector species, regrading streams and even straightening river banks may reduce vector populations (Thevasagayam, 1985). Some of these activities require regular maintenance, whereas others are permanent changes to the landscape (although they may require substantial initial effort to establish). Environmental modification can address the problem of human-made vector breeding sites associated with water-holding structures in mini-dams and small-scale irrigation projects. The creation of favourable vector habitat may be avoided through careful design (W.H.O., 1982).

A number of reviews have evaluated large-scale environmental modification projects included in broad-based malaria control or eradication programmes largely prior to the 1940s in Italy (Fantini, 1998); the Tennessee Valley, U.S.A. (Kitron & Spielman, 1989); Indonesia (then the Dutch East Indies) (Takken *et al.*, 1990); Malaysia (Konradsen *et al.*, 2004); and Zambia (then Northern Rhodesia) (Utzinger *et al.*, 2001). These environmental modification projects contributed to the successful reduction or elimination of mosquito breeding habitat, typically after an initial adjustment period and significant costs (over US$ 1 000 000 in the case of Zambia). In Italy, modification efforts involved substantial investment of money and time to achieve major engineering feats including the 16-km diversion of the Ombrone River into a canal and draining the almost 100 000-ha Pontine marshes using an elaborate series of ditches and canals (Fantini, 1998). Smaller-scale modification projects focused on eliminating coastal habitat for *Anopheles sundaicus* (Rodenwalt) in the Dutch East Indies (Takken *et al.*, 1990) and Malaysia (Konradsen *et al.*, 2004). In the south-eastern U.S.A., large-scale wetland drainage projects implemented as part of the New Deal during the 1930s depression may have contributed to the virtual elimination of malaria from the region by the 1940s (Kitron & Spielman, 1989), although Humphreys (1998) argues that the mass human migration to cities during that period may have been a more important factor in malaria's decline. In Zambia, techniques including bank modification and vegetation clearance along the Luanshya River and draining of swamps, later combined with indoor DDT spraying, enabled the development of the Zambian Copperbelt, and effectively controlled malaria for several decades (Watson, 1953; Utzinger *et al.*, 2001).

Although interest in environmental modification waned with the rise of DDT, the practice is now being re-examined as countries look for more sustainable, less pesticide-intensive approaches to malaria vector control. In Africa, several recent environmental modification projects have involved the renovation of abandoned drainage systems. In Kitwe, Zambia, a project was begun in the 1950s using lined drains, filling and leveling, and planting of eucalyptus and other water-intensive vegetation to convert a large peri-urban wetland into a public park (Baer *et al.*, 1999). New environmental modification projects modelled after this earlier success were begun in 1998 and initial entomological data suggest that this effort reduced adult mosquito densities (Baer *et al.*, 1999). A small study in urban sites in Uganda reported statistically significant declines in malaria parasitemia rates associated with reduction of breeding sites through environmental modification (Lindsay *et al.*, 2004). In urban Dar es Salaam, Tanzania, an integrated malaria control programme operated from 1988 to 1996 found restoration and maintenance of drains to be 'one of the most effective measures' for reducing both vector populations and malaria parasite rates among school children in the urban areas (Castro *et al.*, 2004). Because the drainage system was already in place, the material and operational costs were significantly lower than in other vector control techniques.

The efficacy of environmental modification to reduce or eliminate malaria vector breeding habitats depends both on the initial design and construction of the project as well as regular maintenance. Although some drainage efforts create permanent, self-sustaining changes to the environment, many modification projects require regular maintenance. Poorly maintained drainage projects may actually increase larval breeding habitat (Takken *et al.*, 1990; Castro *et al.*, 2004). Because modification projects tend to require significant initial investments in construction, such a method may only be feasible where the water body or wetland considered is clearly the main larval breeding site for the malaria vector species. Furthermore, as noted by Konradsen *et al.* (2004), environmental modification requires a highly species- and site-specific approach, and techniques applied successfully in one region may be ineffective in another. The successes of environmental modifications in the earlier part of the 20th Century were generally obtained only when implemented in concert with other strategies (Kitron & Spielman, 1989; Fantini, 1998; Utzinger *et al.*, 2001). Modelling exercises based on the concept of mosquito resource availability (Killeen

et al., 2004) suggest that combinations of interventions to reduce the availability both of water sources for oviposition and of human bloodmeals can be much more effective than individual interventions.

Temporary environmental manipulation of mosquito habitat

Environmental manipulation refers to activities that reduce larval breeding sites of the vector mosquito through temporary changes to the aquatic environment in which larvae develop. These techniques may be appropriate either when permanent removal of aquatic habitat through environmental modification is not feasible or, in the case of irrigated agriculture, when the sporadic presence of water is necessary for other activities. Techniques include changing water levels in reservoirs, flushing streams or canals, providing intermittent irrigation to agricultural fields (particularly rice), flooding and/or temporarily draining human-made or (where feasible) natural wetlands, and changing water salinity. Management of vegetation in or around potential breeding sites is also considered a form of environmental manipulation (Rafatjah, 1988).

The feasibility of flushing streams to control *Anopheles culicifacies* Giles was examined in five river systems in rural Sri Lanka in the late 1930s (Konradsen *et al.*, 2004) and again in one stream in the mid 1990s (Konradsen *et al.*, 1998). Stream flushing successfully reduced larval density in cases where local stream and bank conditions facilitated efficient movement of water (Konradsen *et al.*, 2004). Flushing has also been used successfully in Mexico to remove *Anopheles pseudopunctipennis* Theobald larvae associated with rice fields (Rafatjah, 1988).

Intermittent irrigation has been suggested to remedy increased malaria vector abundance associated with irrigated agriculture, specifically rice (Van der Hoek *et al.*, 2001). Irrigated rice cultivation in particular has been clearly linked to increased malaria transmission in areas of Africa and other parts of the world (Surtees, 1970). Intermittent irrigation involves periodic draining of the fields timed at a frequency to prevent mosquito larvae from completing their development cycle. The wet-dry cycles may vary in length from 2–3 days to 2 weeks, depending on rice variety and planting system (Keiser *et al.*, 2002). This method has proven successful in rice growing regions in India, China, and other parts of Asia (Lacey & Lacey, 1990). In China, vector control has also been accomplished by simply letting fields dry up naturally (Pal, 1982), and where it is cold enough for a significant proportion of malaria vector populations to overwinter as larvae, malaria control has been enhanced by combining intermittent irrigation of wet-field crops throughout most of the year with seasonal crop rotation based on planting a dry-field crop in the winter (Liu *et al.*, 2004). The combined use of the natural insecticide neem (*Azadirachta indica*) and intermittent irrigation in Indian rice fields achieved substantial reductions in culicine larvae as well as smaller, but still significant reductions in anophelines (Rao *et al.*, 1995).

An extensive review of large-scale trials of intermittent irrigation in Europe, Asia, Africa and Peru by Keiser *et al.* (2002) found it to be highly effective in reducing vector density in the majority of cases (12 out of 17). In the same trials, rice yields were typically similar to or higher than yields in conventionally irrigated fields (nine out of 12 cases in which rice yield was measured). However, in two of the three African studies cited, the technique was not successful because the soils did not dry out completely and vector breeding continued in remaining puddles (Grainger, 1947, Kenya; Van der Hoek *et al.*, 2001, Tanzania). The third African study by Mutero *et al.* (2000) in Kenya showed a high level of *An. arabiensis* larval mortality, but preferential oviposition by adult vectors in intermittently irrigated fields caused overall vector abundance to remain the same.

Environmental manipulation through management of vegetation has also been implemented to reduce vector breeding populations, either by eliminating aquatic habitat or making it less suitable to vector larvae. For example, planting water-intensive tree species such as *Eucalyptus robusta* can reduce standing water in marshy areas (W.H.O., 1982; Sharma *et al.*, 1986; Sharma & Sharma, 1989; Baer *et al.*, 1999). Vegetation may also be managed to affect light conditions. Such approaches generally require extensive knowledge of the ecology of the local vector population. Malaria experts have long observed that some vector species prefer shaded breeding sites (e.g. *An. umbrosus* in Malaysia) whereas others such as *An. maculatus*, *An. gambiae s.l.*, *Anopheles minimus* Theobald, and *An. sundaicus* prefer sunny conditions (Rafatjah, 1988). Based on this knowledge, early 20th Century programmes to control *An. maculatus* in Malaysia involved preservation or planting of shade trees over breeding sites (Hackett *et al.*, 1938.) Aquatic vegetation, particularly algae, is associated with high larval densities of some malaria vectors, including *An. pseudopunctipennis* Grassi in Latin America (Rejmankova *et al.*, 1993) and *Anopheles subpictus* in India (Rajagopalan *et al.*, 1991). In a controlled field study, Bond *et al.* (2004) found manual algal removal from breeding pools along a river in southern Mexico significantly reduced both larval and adult densities of *An. pseudopunctipennis* for up to 6 weeks. Rajagopalan *et al.* (1991) observed similar results in a community-based programme to remove algae from *An. subpictus* coastal breeding sites in south-east India.

Although more widely used to control mosquitoes of the genus *Culex*, a vector of lymphatic filariasis, expanded polystyrene beads (EPBS) have also been applied to control anopheline larvae in water tanks and abandoned wells, particularly in India (Sharma *et al.*, 1985; Dua *et al.*, 1989; Sharma & Sharma, 1989; Dua *et al.*, 1997). EPBS form a floating layer on the water surface, blocking mosquito oviposition and causing high larval mortality. The advantages of the EPBS method are its simplicity, safety, low cost and persistence. However, the beads tend to blow off of shallow water bodies exposed to wind and may be collected by children, so EPBS may be most suitable for enclosed structures such as cisterns, wells or tanks (Singh *et al.*, 1989). EPBS were applied specifically for control of *Culex* larvae in the integrated urban malaria control programme in Tanzania (Castro *et al.*, 2004). Although these mosquitoes cannot transmit malaria, their management was important for community acceptance of the malaria control programme.

The efficacy of all environmental manipulations depends on several key factors, including how well the intervention is matched to the specific ecological requirements of local vector

mosquito populations. Effective implementation depends on accurate information on the distribution of breeding sites, and establishing ongoing entomological monitoring capacity to modify interventions. Environmental conditions including humidity, rainfall and soil composition also affect interventions on the malaria vector and disease transmission. Interactions with agricultural systems and techniques are critical. For example, in the case of intermittent irrigation, poor soil drainage may result in standing pools that permit vector larvae to complete development, so the technique may only be suitable on sandy soils (Keiser *et al.*, 2002). Finally, as environmental manipulations are aimed at reducing overall vector population density, good coordination of activities at a local level is needed (Lacey & Lacey, 1990), and broad mosquito control is generally likely to be better accepted by participating communities than interventions targeted solely to anopheline mosquitoes (Lindsay *et al.*, 2004).

Secondary and unintended effects of environmental management

The impacts of environmental management depend in part on the scale of the operation. Clearly, some of the large-scale, permanent draining projects, particularly of natural wetlands, must have significant impacts on local ecosystems. Zimmerman & Berti (1994) pointed out that natural wetlands are in decline worldwide and strongly suggested that modification of natural ecosystems be avoided. Conversely, one of the economic benefits of past large-scale draining operations was the creation or renovation of land for agriculture or building construction (PEEM, 1986; Takken *et al.*, 1990; Konradsen *et al.*, 2004). In addition, creating or restoring drainage, sanitation and water storage structures, particularly in urban and peri-urban areas, can reduce the risks of many water-borne diseases, such as diarrhea (Esrey, 1996).

Environmental manipulation through water management can affect access to water for other uses. For methods such as intermittent irrigation to be practical, they should have a minimal or, preferably, positive impact on crop yields, as has been demonstrated in some trials of intermittent irrigation of rice (Keiser *et al.*, 2002). Activities that involve flooding or flushing of streams with high volumes of water could create drowning hazards to people in nearby communities, so safety measures would need to be developed (W.H.O., 1982). Finally, although temporary manipulation activities are not expected to be as disruptive to local ecosystems as permanent modification, dramatic changes in water levels are likely to impact some non-target aquatic organisms.

Applicability of environmental management in Africa

Large-scale drainage and other environmental modifications may be appropriate in some transmission situations in Africa, but programmes to apply such techniques today would probably differ strikingly from those of the early 20th Century. The early environmental modification projects required significant inputs of labour, sometimes through obligatory labour contributions by the communities (Takken *et al.*, 1990). In areas under colonial rule, such as the Dutch East Indies, measures to reduce larval habitat including sealing water sources and the removal of fish ponds were forcibly imposed on communities (Takken *et al.*, 1990). Clearly, top-down, authoritarian approaches are not in keeping with the community-based approaches of governments and non-governmental organizations operating malaria control programmes in Africa today (Konradsen *et al.*, 2004). Also, the large up-front investments of capital and labour would require significant government or donor support, as well as patience in achieving results. The experiences in Zambia, Uganda and Tanzania, however, suggest that environmental modifications, such as land drainage, might be both acceptable and cost effective in African urban and peri-urban areas, particularly if old drainage systems exist that may be restored and maintained (Utzinger *et al.*, 2001; Castro *et al.*, 2004; Lindsay *et al.*, 2004).

Some environmental manipulation techniques may be applicable to African malaria transmission conditions, but others may not. Stream flushing is not suitable to the larval ecology of the main African vectors. Vegetation management might be applicable, either shading to control *An. gambiae s.l.* or removal of emergent vegetation to control *An. funestus*, but does not appear to have been explored at an operational level. As acreage of irrigated rice increases in Africa, interest in vector control through intermittent irrigation may grow. The results of initial trials, however, are not promising (Grainger, 1947; Mutero *et al.*, 2000; Van der Hoek *et al.*, 2001). A further complication limiting the feasibility of African malaria vector control through intermittent irrigation may be resistance to dessication for 2–12 days observed in both laboratory and field studies of *An. gambiae s.s and An. arabiensis* eggs (Beier *et al.*, 1990; Koenraadt *et al.*, 2003). A recent study in Mali by Klinkenberg *et al.* (2002, 2003) suggests that the main factors contributing to increased *An. gambiae s.s.* density in irrigated rice were asynchronous planting schedules and the practice of leaving fallow fields flooded. If these practices are not necessary for agronomic reasons, environmental management of malaria vectors in rice fields may be possible through coordinated planting and better drainage, without requiring more complicated intermittent irrigation methods. It should be noted that, although irrigated rice can increase malaria transmission in Africa, this is not always the case (e.g. Burkino Faso; Service, 1989). Also, even if vector populations increase, irrigated rice projects may provide increased financial resources for malaria prevention and treatment, thus lowering the overall social burden of the disease (Ijumba & Lindsay, 2001). Given these issues, the potential role of environmental manipulation for control of African malaria vectors is not clear. Further studies of vegetation management and irrigation strategies would be useful.

Larviciding: biological and chemical methods

Although environmental management involves physical changes to the mosquito larval breeding habitat, mosquito suppression can also be achieved through treating the breeding sites directly

with chemical or biological agents that kill the larvae. This approach may be appropriate when environmental management techniques such as permanent or temporary drainage of water are not possible or desired. Larviciding is feasible and effective when breeding sites are relatively few in number and/or are easily identified and treated. Therefore, some vector species, such as the Indian vector *Anopheles stephensi* Liston, whose larvae are generally restricted to human-made water containers in urban areas, may be particularly good targets for such control methods. Chemical larviciding and biological control, particularly using larvivorous fish, were important to malaria control programmes in the early part of the 20th Century, particularly in cities (Gratz & Pal, 1988). Larviciding also played the primary role in the eradication of *An. gambiae s.l.* from rural Brazil in the 1930s (Killeen *et al.*, 2002b).

Chemical larvicides

The goal of chemical larviciding is to eliminate or reduce the vector population by killing the larvae. Chemical larviciding was commonly used prior to the commercialization of DDT, particularly for control of malaria in urban and peri-urban areas (Gratz & Pal, 1988). In addition, it has been widely practiced to control nuisance-biting mosquitoes, particularly in the U.S.A (FCCMC, 1998). Because some chemical larvicides may be toxic to non-target organisms, it may not be advisable to apply those compounds to natural water bodies of environmental importance. Due to their low mammalian toxicity and short environmental persistence, certain larvicides such as temephos are applied to drinking water sources for vector control in some countries, but in countries such as the U.S.A., the application of any chemical larvicide to drinking water is prohibited (USEPA, 2002; 2003).

A range of chemicals has been used successfully as malaria vector larvicides. Petroleum oils were applied over 100 years ago to asphyxiate larvae of malaria vectors and other mosquitoes (Gratz & Pal, 1988). The larvicidal poison Paris Green (copper acetoarsenite) was employed against anophelines extensively until the 1940s, by application as a fine powder that floated on the water surface where it was eaten by *Anopheles* larvae (Rozendaal, 1997). Systematic use of Paris Green over approximately 54 000 km^2 of apparently ideal habitat in northeast Brazil during the 1930s contributed to elimination of *An. gambiae s.l.* from this region where it had been accidentally introduced (Killeen *et al.*, 2002b). Although inexpensive and highly effective, use of Paris Green is no longer recommended, considering the risks posed by its high toxicity and to the availability of safer alternatives (Coosemans & Carnevale, 1995). Heavy petroleum oils have also been replaced with lighter, less messy products such as monolayer surface films which may show good efficacy against anophelines under certain conditions (e.g. Karanja *et al.*, 1994). DDT was an early alternative larvicide in the 1940s and 1950s, but is also no longer recommended for larval control or any outdoor applications due to its high persistence and non-target effects. In addition, the use of DDT as a larvicide could undermine the effectiveness of DDT as an adulticide through selection pressure for resistance. Several organophosphate-based larvicidal formulations were developed in the 1960s. In particular, temephos, exhibits very low mammalian toxicity (Gratz & Pal, 1988; FCCMC, 1998) and has been used routinely for malaria vector control in several countries including India, Mauritius and Oman (Kumar *et al.*, 1994; Gopaul, 1995; Parvez & Al-Wahaibi, 2003, respectively). Synthetic pyrethroids are also effective but are problematic as larvicides due to their high toxicity to aquatic non-target organisms (Chavasse & Yap, 1997; W.H.O., 2006). Also, pyrethroids are important as bednet treatments for adult vector control, and the use of pyrethroids as larvicides could spur vector resistance to these valuable insecticides. Insect growth regulators (IGRs) are especially recommended due their greater persistence, the apparent lack of non-target effects and the low probability of cross-resistance with older compounds used as larvicides (Graf, 1993).

Several pilot projects involving larviciding with IGRs show promising results. In Thailand, applications of pyriproxyfen at a rate of 0.005 mg/l to slow-moving rivers significantly reduced adult populations of several vector species, including *An. minimus* and *An. maculatus* (Kanda *et al.*, 1995). In a gem-mining region in Sri Lanka, where the malaria vectors *An. culicifacies* and *An. subpictus* breed in small pits left by miners, Yapabandara *et al.* (2001) found that applications of pyriproxyfen at a rate of 0.01 mg/l three times a year controlled the two vector species and reduced malaria incidence by 75%. In the integrated urban malaria control programme in Dar es Salaam, Tanzania, larviciding with an unspecified IGR was identified along with drain maintenance as the most cost-effective and sustainable vector control method (Castro *et al.*, 2004).

The efficacy of chemical larviciding depends on several factors including the formulation, water quality, and the susceptibility of the targeted larvae. Larvicidal activity tends to be short for conventional fluid or wettable powder formulations (one to two weeks in the tropics) and can be improved through application of slow-release granules, briquettes, or microencapsulated forms (Rozendaal, 1997). Activity tends to be longer in cooler, cleaner water (Chavasse & Yap, 1997; W.H.O., 2006). Larval resistance to some of the more widely applied larvicides such as temephos is also a growing problem (Majori *et al.*, 1986; Coosemans & Carnevale, 1995).

Biological control

Many organisms help to regulate *Anopheles* populations naturally through predation, parasitism and competition. Biological control refers to the introduction or manipulation of these organisms to suppress vector populations. At present, the main biological control agents that have been successfully employed against *Anopheles* are predators, particularly fish, and the bacterial pathogens *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* that attack the larval stages of the mosquito (Das & Amalraj, 1997). Several fungal pathogens in the genera *Metarhizium* and *Beauveria*, show promise as larvicides (Scholte *et al.*, 2003), although commercial formulations are not presently available for mosquito control (Scholte *et al.*, 2004). Other possible biological controls include the nematode

Romanomermis culcivorax and the aquatic plant Azolla (Lacey & Lacey, 1990).

The advantages of biological larval control agents in comparison to chemical controls can include their effectiveness at relatively low doses, safety to humans and non-target wildlife (including natural predators of mosquito larvae), low-cost of production in some cases, and lower risk of resistance development (Yap, 1985). In some cases, low toxicity and simple application procedures make certain biological larval controls particularly appropriate for community-based malaria prevention programmes because community members, even children, may safely apply such products. However, biological control agents tend to be more specific in terms of which mosquito species they can control and in which habitats (Das & Amalraj, 1997). The short persistence of activity of some biological agents often requires repeated applications, which increases costs and logistical complications.

Microbial control. Two bacterial species, *B. thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs), have been widely demonstrated to be effective larvicides against mosquitoes, including anophelines, and various other nematoceran Diptera with aquatic larvae [e.g. Ceratopogonidae (biting midges), Chironomidae (non-biting midges) and Simuliidae (blackflies); de Barjac & Sutherland, 1990]. Both Bti and Bs function as stomach poisons in the mosquito larval midgut. Since the discovery in 1977 of the mosquito larvicidal activity of Bti (serotype H-14), several formulations of Bti have been developed for use against certain types of mosquito larvae, including important malaria vectors such as *An. albimanus*, *An. sinensis* and *An. stephensi* as well as members of the *An. gambiae*, *An. maculatus*, *An. maculipennis* and *An. sundaicus* complexes (Lacey & Lacey, 1990; Becker & Margalit, 1993; Das & Amalraj, 1997; Mittal, 2003). The lethal effect of Bti on mosquito larvae is largely due to protoxins in parasporal crystals and the spore coat, rather than the actual infection. For environmental safety therefore some formulations use dead spores that do not reproduce or persist in the field. Bti is usually active for one to two weeks at most (Lacey & Lacey, 1990; Mittal, 2003). By contrast, Bs (Serotype H 5a 5b) formulations tend to use live spores and have some capacity to persist and recycle in the field (Des Rochers & Garcia, 1984; Karch *et al.*, 1992). The recycling capacity of Bs may help to explain the longer duration of its larvicidal activity observed in the field (up to 4 months) (Castro *et al.*, 2000; Castro *et al.*, 2002). Bti and Bs also differ in their response to water quality: Bti generally requires fairly clean water to be effective whereas Bs can be used successfully in water with some organic pollution (Rishikesh *et al.*, 1988; Mittal, 2003).

An important advantage of microbial larvicides over most chemical larvicides is the low risk to human health and the environment posed by the microbial products. Bti and Bs toxins do not typically persist or accumulate in the environment or in body tissues and are not toxic to vertebrates and most non-target aquatic organisms (W.H.O., 1999). Therefore, these products can be safely applied in and around human habitations (Siegal & Shadduck, 1990) and in environmentally sensitive areas (USEPA, 2002). However, it should be noted that, as an extra pre-caution, the U.S.A. Environmental Protection Agency does not permit the application of microbial larvicides to drinking water in the U.S.A.

Bti is an important part of mosquito control in the U.S.A. (Lacey & Lacey, 1990; FCCMC, 1998), but it is not part of large-scale routine malaria control operations in other countries. Several recent field trials and pilot control programmes using Bti for malaria vector control are summarized in Table 2. Studies are described as field trials if larvae are exposed to microbial agents under natural field conditions, but on a small scale. Pilot projects are studies conducted at sufficiently large scales to detect population-level effects on vector density and, in some cases, effects on malaria transmission. Fields trials of Bti to control *An. gambiae s.l.* larvae in Africa generally have shown good control, but a short duration of efficacy (Karch *et al.*, 1991; Romi *et al.*, 1993; Fillinger *et al.*, 2003). For example, Bti granules gave good initial control of *An. gambiae s.l.* larvae in irrigation ponds in peri-urban Kinshasa, Zaire (Democratic Republic of Congo, D.R.C) but the residual activity was too short (less than 5 days) to provide reliable control (Karch *et al.*, 1991). Trials of Bti granules for *An. arabiensis* control in three sites in Eritrea were more promising, showing good control of larvae for 2–3 weeks in all sites except streams and pools with high algal content (Shilulu *et al.*, 2003). Pilot projects in other parts of the world also showed favourable results. Projects in rural Ecuador and Peru found that weekly applications of Bti reduced the anopheline biting rate by as much as 70%, although the impact of this reduction on malaria transmission was not measured (Kroeger *et al.*, 1995). In a coastal village in Goa, India, Kumar *et al.* (1998) combined the use of the native fish *Aplocheilus blockii* (Arnold) (Cyprinodontiformes: Aplocheilidae, Dwarf panchax) in large breeding sites (wells and water tanks) and Bti in smaller habitats. Malaria transmission rates in the area receiving the fish and microbial biological control agents were significantly lower than in neighbouring areas that received by means conventional vector control of indoor house spraying with DDT.

Recent field trials of Bs against anopheline larvae in Africa, as well as pilot projects around the world, are summarized in Table 3. African field trials with Bs flowable (Nicolas *et al.*, 1987), or granular formulations, generally provided good control of anopheline larvae for 1–2 weeks (Karch *et al.*, 1991; Skovmand & Baudin, 1997; Skovmand & Sanogo, 1999; Fillinger *et al.*, 2003; Shilulu *et al.*, 2003), although Romi *et al.* (1993) found the duration to be less than 5 days in rural Madagascar. Nicolas *et al.* (1987) found that viable spore concentrations from 1×10^2 to 2×10^3 were required for elimination of *An. gambiae s.l.* larvae from small pools in Burkina-Faso. Trials conducted within the last 5 years tended to find greater persistence than older trials, possibly due to improved formulations, such as the new water-dispersible granular formulations (Fillinger *et al.*, 2003). In a pilot project in urban Maroua, Cameroon, Barbazan *et al.* (1998) found that a large-scale Bs spray programme targeting *Culex quinquefasciatus* delayed the onset of the seasonal malaria transmission period by two months, apparently through impact on anophelines. In a pilot project in a peri-urban village near Kinshasa, D.R.C., Karch *et al.* (1992) found that biweekly applications of Bs granules to

Table 2. Field trials of *Bacillus thuringiensis israelensis* (H-14) against malaria vectors. Trademark (manufacturer): Bactimos (Solvay Duphar, Weesp, Netherlands); Bactoculicide = Bacticide (Sibbiopharm, Berdsk, Novosibirsk, Russia); Teknar (originally Sandoz, Basel; now Valent Biosciences, Libertyville, IL); VectoBac (originally Abbott Laboratories, North Chicago, IL; now Valent Biosciences). Formulation: G, granule; TP, technical powder; WDG, water-dispersible granules; WP, wettable powder; 12AS, flowable, active strength 1200 ITU/mg (international toxic units per mg).

Malaria vector target species	Habitat: country	Product applied (trademark and formulation)	Effective application rate (unit/ha water surface)	Duration of control (days between treatments)*	Reference
Field trials					
An. gambiae s.l.	Irrigation ponds: suburban Kinshasa, Zaire	VectoBac-G	10–20 kg/ha	Less than 5 days	Karch *et al.* (1991)
	Open plastic tubs: Lake Victoria, Kenya	VectoBac WDG	0.2–1.6 kg/ha	2–4 days	Fillinger *et al.* (2003)
An. arabiensis	Natural pools, ricefields, ditches: highlands, Madagascar	Vectobac 12AS and Vectobac G	0.6–1 L/ha (12AS), 2–10 kg/ha (GR)	Less than 5 days	Romi *et al.* (1993)
	Ditches, stream beds, ponds: Eritrea	Vectobac G	5.6 and 11.2 kg/ha	2–3 weeks	Shilulu *et al.* (2003)
An. stephensi	Contruction sites and tanks: India	Bactoculicide	10 kg/ha	3–7 days	Kumar *et al.* (1995)
An. culicifacies and *An. fluviatilis*	Water tanks and domestic ponds: Uttar Pradesh, India	Bactoculicide	5 kg/ha	Up to 2 weeks (tanks), not effective in ponds	Shukla *et al.* (1997)
An. albimanus	Irrigation ditches, ricefields, ponds, streams: periurban Comayagua, Honduras	Teknar (alone and with chemical larvicides)	1.17 L/ha (Teknar alone)	10 days	Perich *et al.* (1990)
Pilot projects					
An. stephensi	Wells and tanks: Goa, India	Powder	10 kg/ha	1 week	Kumar *et al.* (1998)
An. albimanus	Ricefields and ponds: rural Peru and Ecuado	Vectobac TP and Bactimos WP	1 kg/ha and 2 kg/ha, respectively,	7–10 days	Kroeger *et al.* (1995)

*All field trials listed achieved 88–100% larval mortality within the first 48 h after treatment except Shukla *et al.* (1997), where initial larval mortality varied from 70–96.8%.

Table 3. Field trials of *Bacillus sphaericus* (Strain 2362) against malaria vectors. Trademark (manufacturer): GRISELEFS (Labiofam, Havana, Cuba); Spherimos (Novo-Nordisk, Bagsvaerd, Denmark); Spherix (Sibbiopharm, Berdsk, Novosibirsk, Russia; 'suspension' (Solvay Duphar, Weesp, Netherlands; Nono-Nordisk, Bagsvaerd, Denmark); Vectolex (originally Abbott Laboratories, North Chicago, IL; now Valent Biosciences). Formulation: FC, flowable concentrate; G, granule; TP, technical powder; WDG, water-dispersible granules.

Malaria vector	Habitat: Country	Product applied (trademark, formulation)	Effective application rate (unit/ha water surface)	Duration of control (days to retreatment)*	Reference
Field trials					
An. gambiae s.l.	Irrigation ponds: suburban Kinshasa, Zaire	Vectolex-G	10–30 kg/ha	5–7 days	Karch *et al.* (1991)
	Ponds/rural village: Senegal	Spherimos FC, granules	30 L/ha for FC, 30 kg/ha for granules	5 days (FC), 15 days (granules)	Skovmand & Baudin (1997)
	Rain puddles: Ouagadougou, Burkina Faso	Spherimos FC, granules	30 L/ha for FC, 30 kg/ha for granules	10 days for both forms	Skovmand & Sanogo (1999)
	Open plastic tubs: western Kenya	Vectobac WDG and Vectolex TP	1 and 5 kg/ha	Up to 11 days	Fillinger *et al.* (2003)
An. arabiensis	Natural pools, ricefields, ditches: highlands, Madagascar	Vectolex-G granule	2.5–18 kg/ha	Less than 5 days	Romi *et al.* (1993)
	Ditches, stream beds, ponds: Eritrea	Vectolex-G	11.2 and 22.4 kg/ha	2–3 weeks	Shilulu *et al.* (2003)
Pilot projects					
An. gambiae s.l.	Swamps, rice fields: suburban Kinshasa, Zaire	Vectolex-G	10 kg/ha	7 days	Karch *et al.* (1992)
	Ditches, puddles, flooded areas: periurban Maroua, Cameroon	'Suspension' (Solvay and Novo-Nordisk)	10 kg/ha	Not measured	Barbazan *et al.* (1998)
An. stephensi	Man-made water containers: Panaji City, India	Spherix suspension	10 kg/ha	7 days	Kumar *et al.* (1994)
An. albimanus	Dry season breeding sites: rural Escuintla, Guatemala	GRISELEFS liquid	10 mL/m^2	Up to 4 months	Castro *et al.* (2000)
	Various habitats: Honduras	GRISELEFS liquid	10 mL/m^2	Up to 4 months	Castro *et al.* (2002)

*All field trials listed achieved 90–100% larval mortality within the first 48 h after treatment.

rice fields and swamps caused a 13.6% decrease in the average number of *An. gambiae* bites to humans. Although this reduction was too low to consider the Bs as a successful control by itself, it suggests that Bs may be useful in some integrated control programmes. Pilot projects in India (Kumar *et al.*, 1994) and Central America show even more promise (Castro *et al.*, 2000; Castro *et al.*, 2002). A large-scale trial of weekly applications of Bs in Panaji City, Goa, India achieved significant reductions in both *An. stephensi* density and malaria incidence (Kumar *et al.*, 1994). In Guatemala and Honduras, Castro and colleagues found GRISELESF, a Cuban formulation of Bs, provided good control of *An. albimanus* Weid.larvae for up to 4 months during the dry season (Castro *et al.*, 2000, 2002). In both countries, malaria cases declined by 50% during the year of the larval control intervention. Although the experimental designs did not include untreated control areas for comparison, the results suggest larval control with Bs played a major role in the observed reduction of disease.

Several field studies compared the efficacy and persistence of Bti and Bs under African conditions (D.R.C., Karch *et al.*, 1991; Madagascar, Romi *et al.*, 1993; Burkina Faso, Skovmand & Sanogo, 1999; Kenya, Fillinger *et al.*, 2003). In Burkina Faso and the D.R.C., Bs granules were generally found to be more effective than Bti formulations against *An. gambiae s.l.* whereas, in Madagascar, Bti granules were effective against *An. arabiensis* in a wider range of larval habitats than Bs. Fillinger *et al.* (2003) found new water-dispersible granule formulations of both Bti and Bs to be more effective than powder formulations against *An. gambiae s.l.* in artificial breeding sites tested in western Kenya, with Bs activity lasting up to 2 weeks.

Many environmental factors can reduce the efficacy or effective lifespan of Bti and Bs products. Natural breakdown or inactivation processes are accelerated by heat, ultraviolet light, and water with high organic matter (Lacey & Lacey, 1990; Consoli *et al.*, 1995). Bti and Bs products may also fail to control anopheline larvae due to the tendency of spores to sink below the surface level at which larval feeding occurs (Kroeger *et al.*, 1995; Orduz *et al.*, 1995). Biological control methods at present have significant logistic demands, with weekly applications often recommended to deal with the problem of their short effective lifespans. Such frequent applications may not be economically or operationally feasible in some circumstances (Kumar *et al.*, 1995). Improved slow-release formulations may help to solve this problem. At the same time, researchers are exploring the possibility of genetically modifying other bacteria common in mosquito breeding sites to produce Bti or Bs toxins (Orduz *et al.*, 1995) and combining BS with Bti toxin production in recombinant strains (Federici, 2005; Park *et al.*, 2005).

Countries manufacturing commercially available formulations of Bti and Bs include Canada, China, Cuba, India, Russia and the U.S.A., and the first production facility in Africa has been installed by the International Centre for Insect Physiology and Ecology (ICIPE) at Nairobi, Kenya (Herren & Maniania, 2005). In addition to liquid and wettable powder formulations that are similar to many chemical insecticides, Bti and Bs products available or under development include slow-release granules and briquettes (Chavasse & Yap, 1997; W.H.O., 2006). In Burkina Faso, researchers have experimented with local production of slow-release granular formulations using imported bacilli (Skovmand & Baudin, 1997; Skovmand & Sanogo, 1999). The capacity to produce Bti and Bs locally and economically would make microbial control of larvae more widely feasible. In Peru, communities have used Bti cultured in coconuts to control malaria vectors breeding in fish farm ponds (Ventosilla *et al.*, 1999).

Larvivorous fish. Predatory fish (particularly in the family Cyprinodontidae) that eat mosquito larvae, particularly in the family Cyprinodontidae, have been used for mosquito control for at least 100 years (Meisch, 1985). Prior to the 1970s, the most commonly used species was the mosquito fish, *Gambusia affinis affinis* (Baird & Girard) (Cyprinodontiformes: Poeciliidae), a freshwater species native to the south-eastern U.S.A. This species was introduced widely around the world. The practice has since been discouraged as the efficacy is highly variable and negative impacts of this voracious and aggressive fish on native fauna have been quite significant (W.H.O., 1982). The introduction of *Gambusia* has actually led to the elimination of native fish from certain habitats (Rupp, 1996). More recently, researchers have evaluated native fish species to identify appropriate local biological control agents (Ahmed *et al.*, 1988). A major factor determining the efficacy of larvivorous fish is the suitability of the fish species to the water bodies where the vector species breeds. This issue is best addressed by finding a native fish species that thrives under the conditions present in breeding sites rather than to change breeding sites to suit the fish. In spite of widespread recommendations for the use of fish and extensive laboratory data, however, reports of controlled field experiments evaluating the effectiveness of larvivorous fish in reducing malaria transmission are limited.

Fish may be useful in controlling malaria vectors associated with rice fields (Lacey & Lacey, 1990) (Table 4). In Asia, introduction or management of larvivorous fish has been effective where pisciculture provides additional economic, agricultural, and nutritional benefits (Gupta *et al.*, 1989; Wu *et al.*, 1991; Victor *et al.*, 1994). In China, Wu *et al.* (1991) found that stocking rice paddies with edible fish such as carp improved rice yield, supported significant fish production, and greatly reduced the number of malaria cases. In their review of mosquito control in rice fields, however, Lacey & Lacey (1990) noted that the use of pesticides and fertilizers can negatively impact fish stocked in irrigated fields. Also, the efficacy of fish as mosquito predators may be strongly influenced by aquatic vegetation, which interferes with fish feeding and may provide refuge for the mosquito larvae. Therefore, periodic vegetation removal may be needed to facilitate the activity of the fish (Dua & Sharma, 1994).

Larvivorous fish also show promise in controlling malaria vectors in human-made containers, particularly in urban areas. Fish have been used in both Africa and India to control vectors that breed in human-made water holding structures such as wells, cisterns, and barrels (Table 4). In an urban area in Ethiopia, Fletcher *et al.* (1992) found that the indigenous fish, *Aphanius dispar dispar* (Day) (Cyprinodontiformes: Cyprinodontidae, Arabian pupfish), effectively suppressed *An. culicifacies adenensis* breeding in wells and containers, although the experimental design did not allow the researchers to

Table 4. Large-scale trials of larvivorous fish to control anopheline larvae.

Fish species	Anopheles species targeted	No. fish/m^2 water surface	% Reduction *Anopheles* larval density (all species)*	Duration of control	Release habitat/region	Reference
Aphanius dispar (*indigenous*)	*Anopheles culicifacies adenensis*	Variable	97% (95%)	2–4 weeks	Cisterns, wells: Assab, Ethiopia	Fletcher *et al.* (1992)
Aplocheilus blockii (*indigenous*)	*Anopheles stephensi*	5	75%†	Single intro. (18 months)	Wells, tanks: Goa, India	Kumar *et al.* (1998)
Gambusia affinis (*introduced*)	*Anopheles stephensi*	Not reported	98%	4 weeks	Wells: Pondicherry town, India	Menon & Rajagopalan (1978)
	Anopheles subpictus, *culicifacies*, *annularis*, *nigerrimus*	5	(86%) Not reported	42 days (study duration)	Ricefields: Uttar Pradesh, India	Das & Prasad (1991)
Oreochromis spilurus spiluru (indigenous)	Anophelines (not identified to species)	1 fish per 3–4 m^2	53%	Single intro.	*Berkits* (cement water tanks): Somalia	Mohamed (2003)
Poecilia reticulata (introduced)	*Anopheles gambiae s.s.*	3–5	85%	Single intro. (1 year)	Basins, cisterns: Grande Comore Is.	Sabatinelli *et al.* (1991)
	Anopheles stephensi and *subpictus*	5–10 per container	(81–86%)	Variable	Containers: India	Gupta *et al.* (1992)
	Anopheles stephensi	5–10	(78%)	Variable	Wells: India	Rajnikant *et al.* (1993)
	Anopheles stephensi	10	97%	4 weeks (study duration)	Human-made containers: Ahmedabad, India	Chapman (2000)
Combination: *Cyprinus carpio* L. (Cypriniformes: Cyprinidae, Common carp), *Ctenopharyngodon idella* (Valenciennes) and other edible species	*Anopheles sinensis*	1	Significant reductions	170 days (study duration)	Rice fields: China	Wu *et al.* (1991)
	Anophelines (not identified to species)	Not reported	81%	Not reported	Rice fields: southern India	Victor *et al.* (1994)

*Percentage decline measured by the change in percentage of possible breeding sites infested with anopheline larvae after fish were introduced.
†Compared average percentage of breeding sites with *An. stephensi* larvae during 6-month period prior to fish release with average percentage of infested breeding during the same 6-month period in year following release.

assess the impact on malaria transmission. In northern Somalia, a locally developed initiative to control water tank-breeding malaria vectors using the indigenous fish *Oreochromis spilurus spilurus* Günther (Perciformes: Cichlidae, River tilapia) found average larval densities reduced by 50% after 1 month (Mohamed, 2003). On Grande Comore Island, where the vector *An. gambiae s.s.* breeds only in human-made reservoirs, the introduced fish, *Poecilia reticulata* Peters (Cyprinodontiformes: Poeciliidae, Guppy), provided year-long suppression of larval and adult mosquito populations and significantly reduced malaria incidence (Sabatinelli *et al.*, 1991). In the majority of the breeding sites on the island, the fish reproduced successfully, thus reducing the need to restock. A number of studies have found that both introduced fish species (*Gambusia affinis* and *Poecilia reticulata*), and indigenous species are effective at suppressing *An. stephensi* populations breeding in containers in India (Menon & Rajagopalan, 1978; Gupta *et al.*, 1992; Rajnikant *et al.*, 1993; Chapman, 2000). A pilot project conducted in Goa, India, combined the use of the native fish *Aplocheilus blockii* in large breeding sites and Bti in smaller habitats and significantly reduced malaria transmission (Kumar *et al.*, 1998).

Urban malaria control programmes using fish must determine whether the community will accept fish swimming in their drinking and bathing water and educate inhabitants to avoid killing the fish accidentally. Researchers have generally found public acceptance of fish to be high (Fletcher *et al.*, 1992; Gupta *et al.*, 1992; Rajnikant *et al.*, 1993; Kumar *et al.*, 1998; Mohamed, 2003), but sometimes investigators encountered individuals concerned about negative impacts of the fish (Sabatinelli *et al.*, 1991). When fish are used in human-made breeding sites in urban areas, periodic restocking is usually necessary to maintain populations sufficient to suppress the mosquito larvae. Malaria control programmes that include fish usually must develop fish hatchery and distribution programmes, particularly for control of container-breeding vectors (Gupta *et al.*, 1989), and this infrastructure can be expensive. Fletcher *et al.* (1992) found that restocking of fish was necessary due to a number of factors, including loss of fish during cleaning or accidental contamination of the container with hot or chlorinated water. The high chlorine tolerance of *Oreochromis spilurus spilurus* made this species suitable for introduction into treated reservoirs in Somalia, but Mohamed (2003) expected restocking to be necessary after the dry season, when water tanks dry up. Larvivorous fish may also need to be supplemented by another larvicidal agent (e.g. one of the Bacillus products) used to control mosquitoes breeding in sites where fish cannot survive (Kramer *et al.*, 1988; Kumar *et al.*, 1998).

Secondary and unintended effects of biological and chemical larval control

Chemical or biological control of anopheline larvae involves the application of the control agents to water bodies. Therefore, the potential for contaminating aquatic ecosystems and even human drinking water is a serious concern. As mentioned previously, microbial larvicides are valued in part due to the low risk of environmental disturbance or human health problems associated with their application. By contrast, early chemical larviciding programmes using products such as petroleum oil, DDT, or Paris green undoubtedly killed many aquatic organisms and may have caused profound changes in certain ecosystems. Today the organophosphate insecticide fenthion is still widely used in spite of its relatively high toxicity to non-target fauna (Rozendaal, 1997). Even temephos (trade name Abate), which is not acutely toxic to mammals, has been found to harm crabs, shrimp, and zooplankton, leading to the requirement in the U.S.A. that this chemical not be applied to environmentally sensitive areas (FCCMC, 1998; USEPA, 2003). Although some of the chemical and microbial products exhibiting low mammalian toxicity have been added to drinking water to control vector larvae in many countries, the application of any such products to drinking water is prohibited in the U.S.A. (USEPA, 2002; USEPA, 2003).

Larvivorous fish have also been used in drinking water, although the issue of possible health impacts has not been widely addressed. The introduction of exotic fish species is associated with the disruption of native fish populations but has been addressed by the move to indigenous fish or fish introduced a long time ago. However, manipulating even native fish into different habitats may have some unintended ecological consequences.

A positive secondary effect of biological control using edible larvivorous fish is the improvement to income and/or diet. The use of edible fish in malaria control may also encourage community support and the long-term sustainability of the programme. However, malaria control programmes that include fish usually must develop fish hatchery and distribution programmes, particularly for control of container-breeding vectors (Gupta *et al.*, 1989), and this infrastructure can be expensive.

Application of chemical and biological controls for malaria prevention in Africa

As the literature reviewed indicates, both chemical and biological larvicides have been applied successfully to control African malaria vectors. These successes suggest that the 50% reduction in vector emergence assumed in the model by Killeen *et al.* (2000) of the combine impacts of ITNs and larval control could be achieved in some situations. Although the reported use of some agents, particularly fish, is limited to transmission situations that are not typical for sub-Saharan Africa, the microbial larvicides Bti and in particular Bs have been tested under a range of conditions against the primary African malaria vectors. Microbial products are effective at controlling larvae, but the duration of activity of present formulations against African vectors is variable and usually requires multiple applications. By contrast, the safety and ease of application of microbial granular formulations, as well as the possibility of local production of these products, suggest they may be appropriate components of community- based, integrated vector control programmes in some African settings. Chemical larviciding with long-lasting IGRs may also be useful as was observed in urban Tanzania (Castro *et al.*, 2004). Given the successful application of the IGR pyriproxyfen to gem pits in Sri Lanka (Yapabandara *et al.*,

2001), similar trials might be conducted to determine whether IGRs would be useful to control *An. gambiae s.l.* breeding in pits left from construction activities as described by Carlson *et al.* (2004) and Fillinger *et al.* (2004).

The growing interest in larval control of African malaria vectors has stimulated debate about the importance of targeting larval control through identification and treatment of those larval habitats producing most adults (Gu & Novak, 2005, 2006; Killeen *et al.*, 2006). Given the sometimes vast numbers of potential breeding sites in rural African communities and the variability in productivity of different breeding habitats (Gimnig *et al.*, 2001; Carlson *et al.*, 2004; Fillinger *et al.*, 2004; Minakawa *et al.*, 2004; Shililu *et al.*, 2003), surveillance and selective treatment of only highly productive breeding sites (targeted control) could be more cost-effective and feasible than general treatment of all possible habitats (untargeted control). A model developed by Gu & Novak (2005) to predict the relative impacts of targeted vs. untargeted larval control on overall vector population densities found that targeted control of just 40% of potential breeding sites could dramatically reduce vector populations and the monthly EIR. However, in their critique of the model, Killeen *et al.* (2006) argued that the level of surveillance precision necessary for effective targeting is neither practical nor biologically useful at an operational level, and point out that untargeted (but exhaustive) larval control successfully eradicated *An. gambiae s.l.* in Brazil and Egypt. Moreover, Gu *et al.* (2006) argued that source reduction causes vectors to spend more time and energy finding suitable sites for oviposition, thus prolonging their gonotrophic cycles and reducing the basic reproductive rate of malaria. Clearly further research and evaluation of large-scale antimalaria programmes that include larval control are needed to resolve this debate.

Community involvement in larval control of malaria vectors

In all larval control methods, the community should be involved, at a minimum in the identification and monitoring of permanent and transitory breeding sites of the malaria vector. In areas where the sites are relatively few and located on land owned by the government, participation in control activities may be limited. In many transmission situations, however, where vectors breed in numerous water bodies or containers located in individual households, community understanding, interest, and involvement are critical to successful larval control programmes (Yap, 1985; Rishikesh *et al.*, 1988). Although large-scale environmental modification efforts would probably need to be planned and coordinated through a government agency, community involvement in all stages from planning to maintenance would increase the sustainability of malaria larval control projects. Use of some techniques, such as chemical larvicides that are perceived to be toxic or oils that are messy may be less appealing to residents than biological agents (Fletcher *et al.*, 1992). In addition, public acceptance and involvement in household larval control programmes may be influenced by the impacts of malaria control interventions on general nuisance biting. In their study of community awareness and mosquito control programmes in two Tanzanian cities, Stephens *et al.* (1995) found that the persistence of nuisance mosquitoes created dissatisfaction with existing insecticide spraying programmes for malaria control. Conversely, the integrated malaria control programme in Dar es Salaam, Tanzania increased community acceptance of the programme through the use of EPBS specifically for control of nuisance-biting *Culex* mosquitoes (Castro *et al.*, 2004). A sociological study of public perceptions of a microbial larvicide-based mosquito control programme in urban Burkina Faso found that people approved of the programme when it caused a reduction in nuisance biting (Samuelsen *et al.*, 2004).

Some larval control methods require significant public involvement. Environmental manipulation through vegetation management, such as algae removal may, be implemented through voluntary community participation (Bond *et al.*, 2004), as was the case in an Indian trial of the technique (Rajagopalan *et al.*, 1991). To effectively suppress vector populations, intermittent irrigation must be practiced over a fairly wide area, requiring a high level of coordination as well as acceptance of the technique by local farmers (Keiser *et al.*, 2002). The deployment of larvivorous fish into human-made containers, particularly water storage tanks, requires extensive community education and involvement, as the fish may be killed easily by householders who do not know how to or do not want to take care of the fish (Fletcher *et al.*, 1992; Gupta *et al.*, 1992; Rajnikant *et al.*, 1993; Kumar *et al.*, 1998; Mohamed, 2003). Applications of Bti or Bs granules or briquettes do not involve special equipment and may be safely conducted by householders or community volunteers (W.H.O., 1999), extending the capacity of often understaffed vector control departments.

Conclusions

This review summarizes how various chemical, biological and environmental management methods can be used to control larvae of *An. gambiae s.l.* in Africa. Although larval controls may not be feasible in transmission situations where breeding sites are too numerous and ephemeral, these techniques may be cost-effective in urban and peri-urban areas. Even in certain rural communities, some form of larval control may be a helpful supplement to indoor residual spraying or insecticide treated nets, particularly during the dry season when vector larvae are concentrated in relatively few breeding sites. Unlike insecticide-based vector control that targets adult mosquitoes, non-chemical larval control, such as environmental management and biological control, pose virtually no risk of environmental contamination and human exposure to pesticides. Non-chemical larval control may also provide a valuable contribution to resistance management programmes, through the prevention or delay in the onset of vector resistance to insecticides used for bednet treatment or indoor residual spraying.

To be implemented effectively, larval control techniques require substantial information about vector ecology, distribution of larval habitats, and local environmental conditions. Successful control in one location may not be predictive of

results elsewhere. At present, there are substantial gaps in the scientific literature, both on control of malaria vector larvae and on the larval ecology of African vectors. Information on *An. funestus* is particularly limited. Published reports describing large-scale implementation of larval control techniques suggest that combinations of several interventions appropriate to local conditions and vectors may provide good control, but such reports are relatively few in number. Furthermore, outcome measures vary between studies, and are often not comparable. More research is needed, particularly large-scale field trials with well-defined measures of efficacy. Despite these limitations, however, this review indicates that interventions against larval anophelines are beneficial in Africa, particularly in conjunction with control measures targeting adult mosquito vectors of malaria.

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