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Community effectiveness of copepods for dengue vector control: systematic review†

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Abstract

OBJECTIVE Vector control remains the only available method for primary prevention of dengue. Several interventions exist for dengue vector control, with limited evidence of their efficacy and community effectiveness. This systematic review compiles and analyses the existing global evidence for community effectiveness of copepods for dengue vector control.

METHODS The systematic review follows the PRISMA statement, searching six relevant databases. Applying all inclusion and exclusion criteria, 11 articles were included.

RESULTS There is evidence that cyclopoid copepods (*Mesocyclops* spp.) could potentially be an effective vector control option, as shown in five community effectiveness studies in Vietnam. This includes long-term effectiveness for larval and adult control of *Ae. aegypti*, as well as dengue incidence. However, this success has so far not been replicated elsewhere (six studies, three community effectiveness studies – Costa Rica, Mexico and USA, and three studies analysing both efficacy and community effectiveness – Honduras, Laos and USA), probably due to community participation, environmental and/or biological factors. Judging by the quality of existing studies, there is a lack of good study design, data quality and appropriate statistics.

CONCLUSION There is limited evidence for the use of cyclopoid copepods as a single intervention. There are very few studies, and more are needed in other communities and environments. Clear best practice guidelines for the methodology of entomological studies should be developed.

keywords dengue, vector control, systematic review, copepods, cyclopoids

Introduction

Dengue is the most rapidly spreading mosquito-borne viral infection in the world today [10]. At present, there are no specific treatments or vaccines for dengue. Vector control is the only method available for primary prevention of dengue targeting the main vector *Aedes aegypti*. A number of innovative interventions have been used, including predatory crustaceans (copepods) which prey on mosquito larvae in standing waters [11].

Copepods are a diverse group of small crustaceans and have been used to control mosquito larvae of public health importance in artificial containers [6, 8, 33]. As other biological control methods, cyclopoid copepods (cyclopoids: a subclass of copepods) are not harmful to human health [11]. The scientific evaluation of the predatory role of copepods started in the 1980s in French Polynesia, where *Mesocyclops aspericornis* induced 99.3% mortality in *Aedes* population. Experiments in Australia and Thailand, particularly those conducted in crab burrows, water tanks, drums and wells, had mixed responses. Copepods also had some degree of success in the Americas, especially in discarded tyre piles [4]. Most

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of the studies mentioned above can be classified as efficacy studies, studies carried out under laboratory/field laboratory conditions.

However, when it comes to community effectiveness – defined, for this systematic review, as studies performed involving communities and/or studies designed to show effectiveness of programmes – several difficulties for the practical application may play a role: copepods generally thrive up to 6 months in containers, but their population is significantly reduced when water dries up or is removed, containers are cleaned, or (copepod) food availability is limited. Consequently, particularly for species without the capacity of dormancy, reintroduction may be needed for sustainable control [7]. There are further operational and financial aspects, such as rearing the organisms on a large scale and their limited use in aquatic sites [11].

Therefore, this systematic review answers the research question: What is the global evidence for community effectiveness of copepods for dengue vector control?

Materials and methods

This review follows the Cochrane Handbook for Systematic Reviews [3], including the PRISMA statement [5]. The AMSTAR measurement tool was used for methodological quality [9].

Six bibliographical online databases were searched: PubMed, EMBASE, Cochrane Database of Systematic Reviews, WHO Library and Information Networks for Knowledge Database (WHOLIS), Web of Science and Latin American Caribbean Health Sciences Literature (LILACS). An exhaustive manual search and a review of reference lists from relevant studies were performed, as well as consultations with experts in the field. The literature search ended on 31 May 2013 with no lower time limit (two data extractors). The category dengue (*Dengue, Dengue H(a)emorrhagic Fever* and *Dengue Shock Syndrome)* was combined with the category for copepods (*copepods* and *Mesocyclops)*, not using Boolean logic operators.

Inclusion criteria were (i) original articles focusing on copepods for dengue control: any language, any time period, regardless of study sites; (ii) community effectiveness studies; and (iii) studies using copepods only.

Exclusion criteria were (i) studies using combinations of entomological interventions (multiple intervention studies); (ii) non-trial studies; (iii) research focus mainly on predatory efficacy; and (iv) studies focussing predominantly on efficacy.

Articles were screened by title and abstract; after applying inclusion and exclusion criteria, the full texts of the remaining articles were retrieved and finally included articles were summarised in a data matrix (evidence table). Scientific quality was assessed; acceptable study designs included cluster randomised trials, intervention control studies and pre- and post-studies. Excluded studies were summarised in an exclusion table.

The data matrix included data/information on baseline characteristics of households and/or communities, geographical characteristics, details of the intervention and control groups, details of the process of administration of the intervention, logistical data and dynamics of community participation. The following entomological indices were extracted to the database: (i) Breteau index; (ii) house index; (iii) container index and (iv) pupae per person [11]. If reported by the studies, adult mosquito indices and human epidemiological parameters were extracted.

Because of the very limited number of studies available, no study was excluded for quality reasons. However, the quality of each individual study is reported in the Results section and considered in the discussion and overall conclusion.

Results

A total of 1222 articles were initially identified. Eleven articles met the eligibility criteria fully (Table 1), after removing duplicates, and the application of inclusion and exclusion criteria (Table 2 for excluded studies). Of these, eight were identified from the published literature (three from reference lists) (see Figure 1 for selection process). Results are also summarised in Table 3.

The majority of the results were generated by PubMed; EMBASE and Web of Science hits were mostly duplicates. Hits on WHOLIS, Cochrane Library and LILACS databases were also either duplicates or not directly relevant to the research question.

Three studies included both efficacy and community effectiveness. Table 1 summarises the studies, including a unique identifier number for further reference in this systematic review. All reviewed articles were published between 1990 and 2012.

Eight community effectiveness studies were found, five of which were conducted in Vietnam (1, 2, 3, 4, 5) by the same group of authors, while the remaining three articles were conducted in the Americas: Costa Rica (7), Mexico (6) and USA (8). Three articles reporting on both efficacy and community effectiveness were geographically located in Laos (9), Honduras (11) and USA (10).

In most studies, the study location was selected on the basis of dengue epidemiology, community health centres, history of dengue epidemics, availability of committed

Table 1 Included studies

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
Community effectiveness studies evaluating the use of Copepods for Dengue Control (n = 8)								
1	[20]	Vietnam (June 2004–April 2010)	Community-Based Control of *Aedes aegypti* by using *Mesocyclops* in Southern Vietnam	Intervention Control study; non-randomised selection of communities. The study aims to apply community-based biological control of *Aedes aegypti* using *Mesocyclops* in southern Vietnam (and to replicate the same successful outcome from northern and central Vietnam provinces).	Community-based programmes were developed in pre-selected communities. Two communities were maintained as untreated controls. Selected communities were mobilised with *Mesocyclops* inoculation into key containers (standard jars >100- to 200-l capacity) which became sites for regular distribution of cyclopoids by collaborators during their monthly visits to each household.	Larval density index (average number of mosquito larvae per house); Adult density index (average number of female *Aedes aegypti* per house on the basis of 15-min aspirator collections of adult mosquitos resting indoors on clothes, walls, under the beds). For disease surveillance, case data were confirmed using an in-house Pasteur Institute of Ho Chi Minh City IgM antibody capture enzyme-linked immunosorbent assay kit	In a total of 14 communities (with 124 743 residents), the mean ± SD density of adult female *Ae. aegypti* was reduced from 0.93 ± 0.62 to 0.06 ± 0.09, and reduction of immature numbers of immature *Ae. aegypti* averaged 98.8%. In the final survey, no adults were collected in 6 of 14 communes. Dengue transmission was reduced in all five communities in which diagnostic serologic monitoring was performed	Conclusion: The study concluded that participation of local authorities and community was a key for success; schoolchildren could be considered as collaborators; a multisectoral approach integrating vertical and horizontal systems and incorporating epidemiologic and entomologic surveillances was necessary in implementing a community programme on dengue prevention and control; inoculation of *Mesocyclops* was considered a good practice because of its inexpensiveness, ease of use, high and sustainable effectiveness.

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
2	[21]	Vietnam (September 2000 – March 2004)	Elimination of dengue by community programmes using *Mesocyclops* (Copepoda) against *Aedes aegypti* in central Vietnam	Intervention control study; non-randomised selection of communities. The programme goals are: (i) to reduce the incidence of dengue and DHF by controlling or eliminating *Ae. aegypti*, and (ii) to strengthen the capacity of health staff at all levels in implementing a community-based programme for dengue vector control using the biological agent *Mesocyclops*.	Community-based control programmes were established in pre-selected communities of Central Vietnam; one community served as untreated control. The intervention consisted of site selection and KAP survey; Establishment of community management committees and collaborator networks; Community training; Involvement of school teachers and students; Baseline *Aedes* and *Mesocyclops* field surveys; Inoculation of *Mesocyclops* into water containers with capacities >100 l; Regular entomologic surveys; Disease surveillance using IgM-capture ELISA.	Larval density index (estimated numbers of third and fourth instars per household); Adult density index; IgM-capture ELISA was used for dengue disease surveillance.	Results showed that from quantitative estimates of third and fourth instars from 100 households, *Ae. aegypti* were reduced by approximately 90% by year 1, 92.3–98.6% by year 2, and *Ae. aegypti* immature forms had been eliminated in two out of three communities by the end of the programme. Similarly, in terms of the resting adult collections from 100 households, densities were reduced to 0–1 per community. Absolute estimates of third and fourth instars at the three intervention communities and one left untreated had significant correlations (P = 0.009–< 0.001) with numbers of adults aspirated from inside houses on each of 15 survey periods The incidence of dengue disease in treated communes was reduced by 76.7% by year 1, compared with non-intervention communes within the same districts	Conclusion: Clinical dengue infections were eliminated in three rural communities. This community-based dengue control strategy using *Mesocyclops* was highly effective in controlling the dengue vector at project sites as indicated by both larval and adult surveys. Key factors influencing the effectiveness of this programme included the attitude and willingness of collaborators, CMCs, school children, local authorities, health staff to target activities at the household level

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
3	[15]	Vietnam (April 1998–March 2000)	Control of *Aedes* vectors of dengue in three provinces of Vietnam by use of *Mesocyclops* (Copepoda) and community-based methods validated by entomologic, clinical, and serological surveillance	Intervention control study; non-randomised selection of communities. The study aims to establish community-based control of *Aedes aegypti* & *Ae. albopictus* using *Mesocyclops* in six communities of northern Vietnam.	Community-based control programmes were established in six pre-selected communities of northern Vietnam; four communities served as untreated controls. Entomological surveys; Training workshops; KAP surveys; *Mesocyclops* inoculation; Clean-up campaigns; Clinical & serological surveillance	Productivity of immature *Ae. aegypti* and *Ae. albopicus* (expressed as a percentage of the numbers of third or fourth instars) was measured in relation to container type frequencies. Measurement of *Aedes* population size and control efficacy was also carried out. Human epidemiological outcome measures include clinical and serological surveillance.	In this study, control efficacy was $\geq$ 99.7%. Although tanks and wells were the key container types of *Ae. aegypti* productivity, discarded materials were the source of 51% of the standing crop of *Ae. albopictus.* The larvae were eliminated from the 3 Nam Dinh communities, and 86–98% control was achieved in the other 3 communities. Variable dengue attack rates made the clinical and serological comparison of control and untreated communes problematic, but these data indicate that clinical surveillance by itself is inadequate to monitor dengue transmission	Conclusion: The control programmes was successful for various reasons: (1) determining key container types which require prioritised control measures; (2) willingness of the community to participate in a health worker-driven system; (3) community recycling projects for economic gain assisted in the removal of small containers unsuitable for *Mesocyclops* treatment; (4) involvement of teachers and schoolchildren; and (5) careful choice of local coordinating committee members and health volunteers

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
4	[22]	Vietnam (February 1993–November 1996)	Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation	Intervention control study; non-randomised selection of communities The study (pilot project) aimed to control *Ae. aegypti* in one village in Vietnam using *Mesocyclops* complemented with community participation (i.e. recycling methods).	One village in northern Vietnam (Phanboi) was the selected site for the pilot project; one village served as untreated control. Study intervention included the inoculation of locally collected copepods by the project staff into the pond-like village well, backyard wells, cement tanks, ceramic jars, and other miscellaneous containers in the village; Initiation of community involvement in copepod distribution; Village-wide recycling programme; Monthly monitoring of copepods and *Ae. aegypti* larvae in containers and adult *Ae. aegypti* inside the houses	Number of larvae and adult *Ae. aegypti* per household	The number of *Ae. aegypti* larvae/house in the treatment village was 30–97% less than the control village during the first 12 months after *Mesocyclops* introduction. In August 1994, the larvae disappeared. No *Ae. aegypti* larvae have been observed in the treatment village since then. The number of adults in the treatment village was 30–100% less than the control village during the first 12 months after introduction of *Mesocyclops*. Adults decreased to 87–99% less than the control village during the 5 months after recycling began, and then they disappeared. Similarly, no adults were detected, representing an absence for 2 years	Conclusion: When used in combination with community recycling, *Mesocyclops* is an easy and inexpensive method of *Ae. aegypti* control that should be effective for many communities in Vietnam and elsewhere Success of the pilot project could be attributed to: (i) the way the villagers used their water-storage containers made it possible to keep copepods in most of the containers with relatively little effort; (ii) community involvement and recycling are helpful in eliminating containers which could not be treated with *Mesocyclops*

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
5	[23]	Vietnam (1995–1996)	Dengue vector control in Vietnam using *Mesocyclops* through community participation	Intervention control study; non-randomised selection of community. The study aimed to determine the effectiveness of using community-based vector control (*Mesocyclops)* for the prevention of dengue fever in Vietnam	A community-based programme was developed in a pre-selected community; one community was maintained as untreated control. Two training courses were organised for project staff on DF/DHF vector control measures using *Mesocyclops*, community participation, and field organising skills. Effectiveness of the methods was assessed by results of KAP surveys, monthly vector surveillance, number of DF/DHF patients, serological surveillance, survival and development of *Mesocyclops* population and by community acceptance	Average adult density index; Larvae house index; Larvae container index; Serological surveillance	Results of vector surveillance showed that *Ae. aegypti* population decreased significantly as compared with control. Post-treatment data showed that the average density index of adult mosquito was 91.5% lower than the pre-treatment phase and was 86.5% ($P = 0.00014$) lower than the control phase. The larvae house index had declined by 64.5% as compared with pre-treatment and decreased by 77.6% ($P < 1 \times 10^{-5}$) compared with the control. The larvae container index was reduced by 68.8% and 72.5% compared with pre-treatment and control village, respectively. There was no clinical dengue case reported in both treated and control communities	Conclusion: This study supports the effectiveness of using *Mesocyclops* and community participation for dengue vector control. The authors concluded that such method is low cost, available locally, easy to be inoculated, released and can survive for a long time. In combination with community recycling, it is found to be an easy and inexpensive method of *Ae. aegypti* control.

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
6	[13]	Mexico (May – August 1994)	Field evaluation of *Mesocyclops longisetus* (Copepoda: Cyclopoidea) for the control of larval *Aedes aegypti* (Diptera Culicidae) in North Eastern Mexico	Intervention control study; non-randomised selection of communities The study aims to evaluate the effectiveness of *Mesocyclops longisetus* in reducing *Ae. aegypti* larval densities in water drums, discarded tyres, and cemetery vases; to determine the persistence of reduction of *Ae. aegypti* larval and pupae stages by an established *M. longisetus* population; and to assess cyclopoid survival and population growth rates in these habitats when helped by community participation	Groups of 200, 50, and 50 *M. longisetus* females were inoculated into 200-l metal drums, discarded tyres, and cemetery flower vases. Larvae were sampled at 15-day intervals and total surviving Cyclops were collected at the end of the study (120 days later). Community participation was solicited through a training programme on copepod rescue before drum cleaning	Effectiveness of mosquito larval control was defined as the percentage of reduction of untreated minus treated containers positive for at least one individual *Ae. aegypti* larva. The per cent reduction formula was computed as [(% untreated container with larvae) – (% treated container with larvae)] ÷ (% untreated containers with larvae) × 100. Copepod control of each larval instar was compared by *t*-tests on arcsine-transformed data from treated against untreated containers	After a period of 4 months, all peridomestic drums still supported variable numbers of cyclopoids. Average of larvae reduction was 37.5% for drums, 67.5% for flower vases and 40.9% for tyres. Despite successful community participation, *M. longisetus* was not able to thrive in high numbers in most of the containers, even in drums where the water was left undisturbed	Conclusion: Sustainable community-based vector control programmes in north-eastern Mexico may result in some degree of success, when copepod populations are released in the field. However, additional ecological factors such as the following have to be met to achieve control: (i) a rich nutrient environment provided by breeding sites; (ii) a high copepod inoculation rate; (iii) a regional rainfall pattern preventing desiccation of temporary larval sites

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
7	[19]	Costa Rica (7 months study duration in 1998)	*Mesocyclops thermocyclopoides* y el control biológico de *Aedes*: ejemplo de un plan de acción comunitaria en Chacarita, Puntarenas/ *M. esocyclops thermocyclopoides* and the biological control of *Aedes*	Intervention control study. The study aimed to determine the effectiveness of using *Mesocyclops thermocyclopoides* as biological control of *Aedes* with the help of community participation	School children were selected and trained regarding the culture and application of copepods in the field. A field experiment was also conducted in a cemetery in which copepods were inoculated in 12 flower vases with water. In addition, the elimination of breeding sites (e.g. discarded containers) was also included. The study lasted for a period of 7 months	There were no clearly defined outcome measures mentioned in the study	Results showed that the copepods survived 2 months in three of the 12 flower vases which were originally inoculated and found to have no mosquito larvae present. On the other hand, five flower vases filled with rain water only (which served as control) contained an average of 100 mosquito larvae	Conclusion: The study concluded that it was possible to get the community participation with respect to biological control and elimination of *Aedes* breeding sites

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
8	[16]	USA (June 1989–June 1990)	Elimination of *Aedes albopictus* from tire piles by introducing *Macrocyclops albidus* (Copepoda, Cyclopidae).	Intervention Control study. The study aims to assess the impact of *Macrocyclops albidus* on mosquito larvae in the tyres and population of adult mosquitoes around the tyres	Three tyre piles with *Ae. albopictus* were established in a wooded area. No maintenance was provided. The only source of water for the tyres was rainfall; thus, some of the tyres dried out from time to time. Each pile had about 15 000 immature *Ae. albopictus*, then 10 adult female *Macrocyclops albidus* were introduced into every tyre in two of the piles. No *Macrocyclops* were introduced to the third pile, which served as control	Larvae, pupae count; adult mosquito landing rate and oviposition	When introduced to two isolated tyre piles, *M. albidus* eliminated all *Aedes albopictus* larvae from both piles within 2 months. Adult *Ae. albopictus* around the tyre piles disappeared within another month. Complete suppression of *Ae. albopictus* larvae was still in effect in all treated tyres a year later	Conclusion: The results of the study suggest that *Macrocyclops albidus* could be considered seriously as a biological control agent for operational use in tyres.

Mixed studies (both Community – effectiveness and Efficacy) evaluating the use of Copepods for Dengue Control (n = 3)

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
9	[14]	Laos (1990–1994)	*Aedes aegypti* control in the Lao People's Democratic Republic, with reference to Copepods	Randomised controlled laboratory trial and Intervention control study (field trial); non-randomised selection of village; with control. The study aimed to explore the potential for integration of *Mesocyclops* spp. as a sustainable, cost-effective tool into the Lao PDR dengue control and surveillance programme.	Copepod collection, identification, laboratory culture, evaluation of predatory and survival abilities, and nutrition trials (performed in Australia); Field culturing of copepods; Field survey to determine the types of containers; Pilot field trial with copepods (release of *Mesocyclops guangxiensis* into 142 containers and 20 wells in a village in Vientiane with one adjacent village as untreated control). *Mesocyclops guangxiensis* was chosen over *M. aspericornis* due to its higher reproduction rate and ability to survive in lower nutrient environments	*Ae. aegypti* larval density (percentage mortality of first instar mosquitos); For the laboratory trials, differences in copepod predation and population growth were tested by analysis of variance or t-test using SAS software. Paired t-tests were used to analyse differences between pre- and post-inoculation population levels of larvae and copepods in the field trials (containers and wells)	One month after the release of *M. guangxiensis*, copepods were present in 7% of the containers and were absent 6 months post-inoculation. In comparison, 100% of wells were still positive for copepods after 6 months, with average numbers of *Ae. aegypti* in wells decreasing from 59.5 ± 18.5 (± SEM) to 0 after 6 months	Conclusion: This study shows that indigenous predacious copepods (*Mesocyclops guangxiensis)* provide control of *Ae. aegypti* in wells, while not in water-storage containers. Overall, the authors concluded that *Mesocyclops guangxiensis* could be integrated as a low-cost, persistent agent into current strategies for improving surveillance and control of dengue vectors

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
10	[17]	USA (3 months study duration in 1990)	Evaluation of Cyclopoid Copepods for *Aedes albopictus* control in tires.	Intervention control study. The purpose of the first laboratory trials was to determine how many first instar *Ae. albopictus* larvae each species of cyclopoid will kill in 1 day. The second laboratory trials aimed to determine: 1) how many *Ae. albopictus* larvae are killed by cyclopoid populations under more natural conditions, 2) the long-term effectiveness of each cyclopoid species as a larval predator, and 3) how the effectiveness is affected by food conditions in a container. In addition to the two laboratory trials, field trials were also conducted	Laboratory and field trials were conducted. In the field trial, ten adult females of *Acanthocyclops vernalis, Diacyclops navus, Macrocyclops albidus, Mesocyclops edax* or *Mesocyclops ruttneri*, as well as mixtures of *Macrocyclops albidus* and *Mesocyclops ruttneri,* were introduced to 600 discarded automobile tyres. Mesocyclops longisetus was introduced to 50 tyres. Approximately 500 of the tyres to which cyclopoids were introduced, plus 150 control tyres, were examined 6–14 weeks after the introductions	*Ae. albopictus* larval count (first instar)	Six to 8 weeks after introduction, *Diacyclops navus, Acanthocyclops vernalis, Mesocyclops ruttneri* and *Mesocyclops edax* reduced the number of *Ae. albopictus* larvae by 83, 90, 95 and 96%, respectively. *Macrocyclops albidus* and *Mesocyclops longisetus* were the most effective species. Six to 8 weeks after introduction, *Macrocyclops albidus* reduced *Ae. albopictus* larvae by 99%. Three months after introduction *Macrocyclops albidus* reduced *Ae. albopictus* larvae by 100%, and *Mesocyclops longisetus* reduced *Ae. albopictus* larvae by 99.8%. *Macrocyclops albidus* and *Mesocyclops longisetus* were equally effective at eliminating *Ae. aegypti* and *Ae. triseriatus* larvae	Conclusion: *Macrocyclops albidus* was the most effective predator; it was the only species that consistently eliminated all *Aedes* larvae. *Mesocyclops longisetus* was also highly effective; predation by *Mesocyclops longisetus* was nearly 100%. *Macrocyclops albidus* and *Mesocyclops longisetus* were also among the species that survived best in tyres.

Table 1 (*Continued*)

Code	Corresponding reference number	Author/s, study location & duration	Title	Study design & objectives	Intervention	Outcome measures	Study results	Study conclusions
11	[18]	Honduras (1991)	Control of larval *Aedes aegypti* (Diptera: Culicidae) by Cyclopoid Copepods in peridomestic breeding containers.	Intervention control Study. The study aims to evaluate the effectiveness of Cyclopoids for the longterm control of *Ae. aegypti* larvae in domestic containers in El Progreso, Honduras. The purpose of the field trials was to determine which containers can be controlled using cyclopoids and which species of cyclopoids are most effective.	Both laboratory predation experiments and field trials were conducted. The latter aimed to compare the survival of each cyclopoid species in different types of containers under normal household use and the effectiveness of each species in eliminating *Ae. aegypti* larvae.	Total number of pupae; larval count	All four cyclopoid species killed > 20 larvae per cyclopoid per day under container conditions. *M. longisetus* was most effective, not only because it was the most voracious predator, but also because it survived best in the containers. *M. longisetus* maintained long-term populations in 200-l drums, tyres, vases, and cement tanks (without drains), provided the cyclopoids were not dried or poured out. *M. longisetus* reduced third- and fourth-instar Ae. aegypti larvae by > 98% compared with control containers without cyclopoids	Conclusion: *M. longisetus* should be of practical value for community-based Ae. aegypti control if appropriate attention is directed to maintaining it in containers after introduction.

Table 2 Summary of excluded articles (n = 21)

Code	Excluded article	Reason for exclusion (not meeting inclusion criteria and/or meeting exclusion criteria)
1	[31]	Multiple intervention study
2	[41]	Morphological study; Descriptive report only
3	[33]	Descriptive report only
4	[27]	Descriptive report only
5	[25]	Non-trial study
6	[30]	Multiple intervention study
7	[32]	Multiple intervention study
8	[34]	Multiple intervention study
9	[40]	Study on predatory potential/efficiency
10	[37]	Study on predatory potential/efficiency
11	[39]	Mainly surveillance and predation study
12	[29]	Multiple intervention study
13	[28]	Descriptive report only
14	[35]	Descriptive report only
15	[26]	Multiple intervention study
16	[44]	Multiple intervention study
17	[42]	Study on predatory potential/efficiency
18	[24]	Study on predatory potential/efficiency
19	[36]	Study on predatory potential/efficiency
20	[38]	Study on predatory potential/efficiency
21	[43]	Study on predatory potential/efficiency

local health staff and community members. No study included an economic analysis.

All 11 studies used study designs comparing study arms applying the intervention to study arms for control. Three studies used communities/villages as unit of allocation for either the intervention or the control (1, 2, 3). In studies 6–11, various breeding containers such as drums, discarded tyres, flower vases and wells were used for either intervention or control. Studies 4 and 5 used the household as unit of allocation. One study looked extensively at the different types of domestic water-storage containers in the study site and analysed the survival and effectiveness of copepods in eliminating *Aedes* larvae in those containers (11). Three studies with mixed study designs (also including an efficacy component) (9, 10, 11) made use of cluster randomised trials in addition to the intervention control design. Studies (9) and (10) focused additionally on the evaluation of the predatory/survival abilities of copepods, as well as nutritional trials.

In the 11 included studies, the copepods used were mostly *Mesocyclops spp.* (study 8 used *Macrocylops albidus*). Sample sizes varied widely. The largest community-based studies evaluated 14 treated communities with two untreated controls (1). The smallest study, on the other hand, examined only one village as the treatment group and another neighbouring village as untreated control (400 households each (4)). Among the efficacy and community effectiveness trials, the smallest study included only 142 containers and 20 wells (9), while the largest study examined approximately 500 discarded tyres with 150 controls (10). The majority of the effectiveness studies provided a limited background of the study sites, including population data, topographical characteristics, access to piped water supply and incidence of dengue. Quantitative assessment of potential confounders (e.g. socio-economic status of the participants, climatic and seasonal factors) was generally lacking in most of the studies.

The main outcome parameters reported in the community effectiveness studies were larval density index and adult density index. One study reported the use of larvae container index, which considered specifically cement tanks, jars and other discarded containers (5). Effectiveness of larval control was defined (6) as the percentage of reduction of untreated minus treated containers positive for at least one individual *Aedes* larva. Another community effectiveness study monitored the adult mosquito population using landing rate and oviposition index (8). Serological surveillance was also carried out in studies 1, 2, 3 and 5. Studies on efficacy and community effectiveness used larval density, pupae and larval counts as outcome measures (9, 10, 11). The majority of community effectiveness studies had clearly defined outcome parameters and accompanying statistical analyses. Detailed discussions on community participation were presented in studies 1, 2, 3 and 5.

Results of studies using communities & villages as unit of measurement

Study (1) is a large-scale intervention control study, evaluating 14 communities with 124 743 residents – two untreated control communities (population of 7149 and 7114). All key containers were treated with cyclopoids by community collaborators. Immature larvae were reduced by 98.8% and adult female *Ae. aegypti* to 0.06 ($\pm$ 0.09). Dengue transmission (serological monitoring) was reduced (χ^2 = 40.0, degrees of freedom (df) = 1, $P < 0.001$) in year 2007, as well as 2 years after (χ^2 = 40.7, df = 1, $P < 0.001$) (Table 3).

In study (2), with an intervention control study design embedded in a community-based dengue control programme, three rural communities with 5913 households and a total population of 27 167 were selected as treated communities (control: one community with 2165

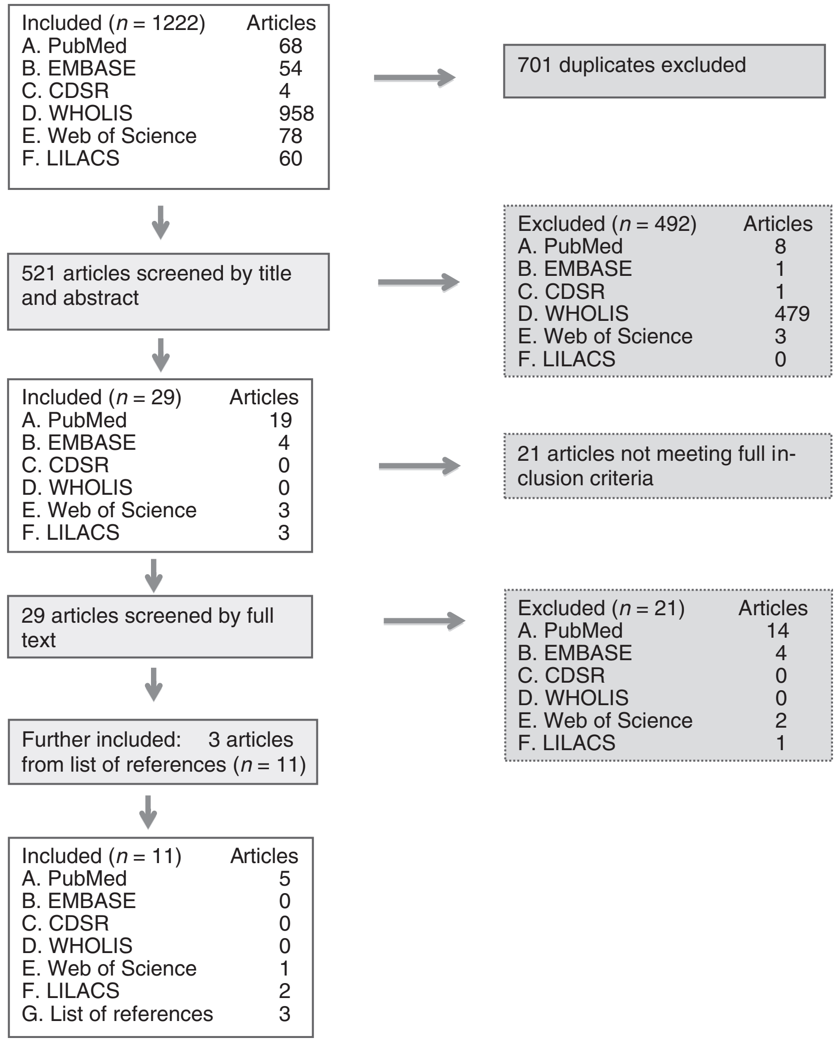

Figure 1 Flow chart of article selection process.

households and 10 419 residents). An initial 90% reduction rate of *Aedes* larvae population was followed by 92.3–98.6% reduction 1 year later. Adult mosquito indices had similar reductions from 0.12–1.16 to 0–0.01 per community after a period of 3 years. The incidence of dengue disease showed 76.7% reduction compared to non-intervention communities (Table 3).

In study (3), also a community-based intervention control study in six intervention communities with 11 675 households and 49 647 residents (four communities as controls with 8909 households and 37 665 residents), efficacy was reported as ≥99.7% in at least three communities. At the end of the study, *Ae. aegypti* larvae population size ranged 0–0.3% compared to untreated communities with 14.4–367.0%. Similarly, *Ae. albopictus* larvae population ranged 0–14.1% compared to 39.1% in untreated communities (Table 3).

Results of studies using households as unit of measurement

Study (4) has one treated and one untreated village (400 households each), reporting 30–97% decreases of *Aedes* larvae per household in the treatment village during the first 12 months. Adult *Aedes* per household in the treatment village diminished by 30–100% during the first year. (Table 3).

Study (5) selected one intervention community with 1600 households and one control community – the latter described to have similar social and environmental conditions, as well as a similar dengue situation as the intervention community. The average density index of adult *Aedes* was 0.05 per house, 91.5% lower than the pre-treatment phase (0.59 per house) and 86.5% lower (P = 0.00014) for the control community. The house index declined by 64.5% compared to pre-treatment levels and 77.6% ($P < 1 \times 10^{-5}$) compared to the control. The

Table 3 Summary of results of included studies ($n = 11$)

Code	Corresponding reference number	Intervention group	Control group
Effectiveness studies using Communities and Villages as Unit of Measurement ($n = 3$)			
1	[20]	The mean ± SD density of adult female *Ae. aegypti* was reduced from 0.93 ± 0.62 to 0.06 ± 0.09, and the reduction of immature numbers of *Ae. aegypti* averaged 98.8%. In the final survey, no adults were collected in six of 14 intervention communities	The two untreated control communities displayed seasonal effects where *Ae. aegypti* abundance was reduced from the end of the wet through the dry season. The larval density index for Tan An community was 149.6 at the start and 67.9 at the end of the survey. For the other control community, Tan Loi Thanh, the larval density indices at the start and end of the surveys were 124.8 and 10.4
2	[21]	The *Ae. aegypti* larval populations in the three intervention communities were reduced by 90% after 1 year and by 92.3–98.6% after 2 years of intervention. Adult indices showed similar reductions from 0.12–1.16 to 0–0.01. *Ae. aegypti* immature forms had been eliminated from two of three intervention communities by the end of the programme	A positive correlation between estimates of log (3rd and 4th instar population size + 1) and the log (mean adults per house +1) *Ae. aegypti* was demonstrated using logistic regression. This was the case for the three intervention localities, Cam Thanh ($P < 0.001$), Binh Chanh ($P < 0.001$), Ninh Xuan ($P = 0.003$) and for the untreated Ninh Binh community ($P = 0.009$)
3	[15]	*Ae. aegypti* population size in six treated communities varied from 0 to 0.3% of the original pre-intervention estimates. 100% control efficacy of *Ae. aegypti* was reported in three communities and ≥ 99.7% control in three other communities. For *Ae. albopictus,* populations in five treated communes varied 0–14.1% from the pre-intervention levels. Pre-intervention: *Ae. aegypti* were found in 137 containers; *Ae. albopictus* were found in 65 containers. Post-intervention: *Ae. aegypti* were found in six containers; *Ae. albopictus* were found in five containers	*Ae. aegypti* population size in two untreated communities varied 14.4–367.0% from the pre-intervention levels, while *Ae. albopictus* population ranged 39.1%. Pre-intervention: *Ae. aegypti* were found in 78 containers; *Ae. albopictus* were found in 14 containers. Post-intervention: *Ae. aegypti* were found in 65 containers; *Ae. albopictus* were found in 13 containers
Effectiveness studies using Households as Unit of Measurement ($n = 2$)			
4	[22]	The number of *Ae. aegypti* larvae/household in the treatment village was 30–97% less than the control village during the first 12 months after *Mesocyclops* introduction. The number of adults/household in the treatment village was 30–100% less than the control village during the first 12 months after intervention. Adults decreased to 87–99% less than the control village during the 5 months after recycling began and then they disappeared. Similarly, no adults were detected, representing an absence for 2 years	
5	[23]	The average density index of adult *Ae. aegypti* in the post-treatment phase was 91.5% lower than the pre-treatment phase and was 86.1% ($P = 0.00014$) lower than the control phase. The larvae house index had declined by 64.5% as compared with pre-treatment and decreased by 77.6% $(P < 1 \times 10)^{-5}$ compared with the control. The larvae container index was reduced by 68.8% and 72.5% compared with pre-treatment and control village, respectively	
Effectiveness and mixed studies using containers as unit of measurement ($n = 6$)			
6	[13]	For drums with regular water use, the percentage of treated drums positive for *Ae. aegypti* was reduced at 31.8%, whereas for drums without water use, there was 46.5% reduction, 43.4% for discarded tyres and 11.3% for flower vases	Control drums with regular water use showed consistent larval populations, with a mean percentage of 56.6% positive over the 4-month study, 78.8% for drums without water use, 75.7% for discarded tyres, and 45.7% for flower vases
7	[19]	Post-intervention: No mosquito larvae were found in 12 treatment containers	Post-intervention: An average of 100 mosquito larvae in five flower vases were present

Table 3 (*Continued*)

Effectiveness studies using Communities and Villages as Unit of Measurement (n = 3)			
Code	Corresponding reference number	Intervention group	Control group
8	[16]	The number of *Ae. albopictus* larvae and pupae in treated piles immediately started to decline 2 weeks after intervention and were nearly gone 2 months post-intervention. None were found in treated piles at the end of the study. *Ae. albopictus* landing rates and oviposition at the treated piles were close to zero 11th week post-intervention	The average number of *Ae. albopictus* larvae and pupae in control pile was more than 200/tyre 2 months after intervention and averaged 43 ± 9.8 (SE) towards the end of the study. Adult landings averaged 2.3 per minute, a total of 138 ± 12 landings, while oviposition at control piles averaged 13 eggs/ovistrip/week, a total of 650 ± 78 eggs towards the end of the study.
9	[14]	Laboratory trials: After 6 weeks, *Ae. aegypti* larvae were absent in containers inoculated with *M. aspericornis* or *M. guangxiensis*. In cages with *M. aspericornis,* the *Aedes* adult population peaked after 2 weeks (152), while in cages with *M. guangxiensis,* adults reached greatest levels after 3 weeks (64). After 7 weeks, no adults remained in inoculated cages. Field studies: After 1 month, *M. guangxiensis* remained present in 7% of the containers but eventually disappeared 6 months post-inoculation. In comparison, 100% of wells were still positive for copepods after 6 months, with average numbers of *Ae. aegypti* in the wells decreasing from 59.5 ± 18.5 (± SEM) to zero after 6 months	Laboratory trials: In the untreated cage, *Ae. aegypti* larvae reached greatest numbers after 2 weeks (1364) but eventually stabilised due to food availability. Adult numbers increased steadily, reaching its maximum (424) at the end of the study. Field studies: In the control wells, the prevalence of mosquito larvae was reduced 20–40% from the first sample to the onset of dry season
10	[17]	Laboratory trials: *Macrocyclops albidus* killed the highest number *Ae. aegypti* larvae (45.0) within 24 h. Larval mortality was near 100% in low food containers and 83% in high food containers. Field studies: The number of *Ae. albopictus* larvae and pupae in tyres with *Macrocyclops albidus* 6–8 weeks after introduction was only 1% of the number in control tyres. *M. albidus* was the most effective predator	Laboratory trials: The average mortality of mosquito larvae in the control group remained low (0.1). Larval mortality in controls was 10% in low food containers and 4% in high food containers. Field studies: The average number of larvae (65.4) and pupae (4.1) in controls 6–8 weeks post-inoculation remained high
11	[18]	Laboratory trials: *Mesocyclops longisetus* killed the highest number of *Ae. aegypti* larvae in culture plates (46.8), drums (36.8) and tyres (36.5) within 24 h Field studies: 17% of the inspections of containers with cyclopoids (n = 468) were positive for first and second instars, while 6% were positive for third and fourth instars.	Laboratory trials: The average mortality of *Ae. aegypti* larvae in controls was 0.1 in culture plates, 18.8 in drums and 14.0 in tyres. Field studies: 38% of the inspections of control containers of all kinds (n = 322) were positive for first and second instars, while 31% were positive for third and fourth instars.

container index was reduced by 68.8% and 72.5% compared to pre-treatment and control. There was no clinical dengue case reported in neither treated nor control communities (Table 3).

Results of studies using containers as unit of measurement

Among community effectiveness studies (6, 7, 8), study (6) reported an average of 37.5% *Aedes* larval reduction in drums, 67.5% in flower vases and 40.9% in tyres. The mean number of total larvae per instar was statistically lower in all types of treated containers than in untreated controls. Cyclopoids significantly reduced total mean larval densities in drums with and without water management (t > 3.34, df = 7, P < 0.05). Similarly, mean density rates were significantly lower in treated tyres and cemetery vases than those without cyclopoids (t > 6.85, df = 6, P < 0.05). This study showed difficulties of using cyclopoids for containers in areas with a prolonged dry season (Table 3).

Study (7), a small-scale study using cyclopoids in flower vases with the participation of schoolchildren, showed that cyclopoids survived for 2 months in three of the 12 inoculated flower vases and that no mosquito larvae were present. Five flower vases that served as control had an average of 100 larvae per container. Outcome measures were not clearly discussed (Table 3).

Study (8) focused on introducing cyclopoids in tyre piles under natural conditions. *Aedes* larvae and pupae in treated tyre piles showed a constant decline during the 4-month period and almost none at the end of the study, whereas the control pile averaged 43 ± 9.8 (Standard Error) immature *Aedes* per tyre at the end of the study. Adult mosquito landing rates and oviposition at the treated piles were close to zero by the 11th week after cyclopoid introduction, while landing rates and oviposition at the control pile started to decline only after *Aedes* population at the treated piles were already close to zero. Adult landing rates at the control pile averaged 2.3 per minute at the end of the study, a total of 138 ± 12 landings. Oviposition at the control pile averaged 13 eggs/ovistrip/week (total of 650 ± 78 eggs). No adult *Aedes,* nor any eggs were collected in the treated piles at the end of the study (Table 3).

The three studies measuring both efficacy and community effectiveness (9, 10, 11) made use of intervention control study design. Study (9) showed for the community effectiveness part of the study that the prevalence of mosquito larvae in the treated containers decreased from 37% to 12% during the study period. Six months post-inoculation, all wells still contained copepods, and the average numbers of *Aedes* larvae had fallen from 59.5 ± 18.5% (± SEM) to zero. In the control wells, the prevalence of mosquito larvae was 20–40% from the first sample until the end of the study (Table 3).

Study (10) focused on comparing six cyclopoid species for biological control of *Aedes* larvae in discarded tyres. Three months after introduction, *Macrocyclops albidus* reduced *Aedes* larvae by 100% and *Mesocyclops longisetus* reduced the larvae by 99.8%. Tyres containing a single species of cyclopoid averaged about one *Aedes* larva per tyre (control tyres had 5.7 larvae per tyre) (Table 3).

Study (11) evaluated the effectiveness of four cyclopoid species in controlling *Aedes* larvae in a variety of containers using laboratory experiments and field trials. *Mesocyclops longisetus* was found to be the most effective in both laboratory and field trials. It reduced *Aedes* larvae by more than 98% compared with control containers. *M. longisetus* was able to successfully maintain large numbers more consistently than other species, particularly in 200-l drums, tyres and vases. The number of immature *Aedes* observed in 200-l drums with *M. longisetus* was less than a thousandth the number in control drums; likewise, the number of immature *Aedes* in tyres with *M. longisetus* was 98% less than in control tyres. No immature *Aedes* were observed in vases with *M. longisetus* (Table 3).

Results concerning larval and adult indices and dengue transmission

Cyclopoid copepods showed excellent results controlling larval *Aedes* populations in treated containers in most studies (Table 3), often with up to 100% elimination of larvae. Only studies 6, 7, 8 and 9 showed less excellent results, but still a reduction of larvae (Table 3). When measuring larval indices at a household level, reductions of households positive for *Aedes* larvae ranged between 30 and 97% (4).

Regarding mosquito landing rates, and also measuring oviposition, reductions to zero were observed in treated tyres (8). Measuring adult *Aedes* per household, reductions were measured between 30% and 100% in the treatment village during the first year (4). Also, adult mosquito indices underwent similar reductions from 0.12–1.16 to 0–0.01 per community after 3 years (2).

In three studies, dengue transmission data were measured: results ranged from zero reported cases in intervention and control communities (5) to a 76.7% reduction of dengue incidence (2) and also a reduction of dengue transmission as measured by serological monitoring (1) (Table 3).

Results concerning delivery of intervention

Community-based studies (1 to 5) discussed community delivery: all five studies had roughly similar elements: Knowledge, attitude and practice (KAP) surveys were undertaken (dengue disease understanding, dengue transmission risk and control options) and repeated regularly, supported by focus group discussions (FGD) and aided by provision of educational material. Study (1) described how community management committees (CMCs) were selected and organised for each village or community. CMCs are usually composed of community health workers, key opinion leaders, local government officials, women's union representatives, youth union representatives, teachers and school children. The CMCs themselves organised a collaborator network of key people per village, and training workshops were conducted (programme objectives and methods, identification of mosquito larvae and adults, cyclopoids, and basic knowledge of dengue). Community members were mobilised starting with the identification and organisation of key containers, cyclopoid inoculation, clean-up campaigns and recycling campaigns (4, 5). Community participation to eliminate discarded water containers and the release of cyclopoids in breeding sites were organised and including home visits, under the steadfast leadership of local authority and health staff (5).

In these studies, the experiences from 'model communities' were expanded into neighbouring villages using local expertise for organisation, mobilisation and local training. Study (1) further discussed the establishment of sustainability funds; proceeds were used to fund the stipends of community collaborators. Study (2) also mentioned the post-project surveillance carried out by the collaborators and CMCs. These activities were particularly important in ensuring sustainability. Community-based dengue control models using cyclopoids have been replicated and expanded in several regions of Vietnam under the national dengue control programme and with some support from external donors. Study (3) further emphasised the importance of gaining coordinated political and communal support in delivering dengue vector control interventions.

In summary, all studies in Vietnam (1–5) reported the delivery of the intervention in detail and success in terms of significant reduction rates of the outcome indicators used, while the rest of the studies reported a relative degree of success.

Discussion

copepods (*Mesocyclops* spp.) could potentially be effective for dengue vector control, as evidenced especially in the context of the large-scale vector control programme in Vietnam (studies 1–5). These community-based studies also showed a successful control of dengue transmission and appear to be sustainable – although the evidence for the control of dengue transmission is limited [7]: these results have not been replicated in other regions [12], as also shown in this systematic review by studies 6–11. Furthermore, the Vietnam-based studies (1–5) were often combined with educational campaigns, and it is difficult to determine whether the copepods alone or the increased awareness about dengue transmission, with related increased protective behaviour of the population, caused the observed effect.

The key factors influencing the positive results of the Vietnam-based studies (1–5) – studies also including positive results for a reduction of dengue transmission (1, 2) – are rigid delivery of intervention, careful selection of community management committees and collaborators, and active participation of community households. The success of these studies has been discussed in the literature and could be 'attributed to a combination of vertical and horizontal approaches in controlling *Aedes* population, efficient identification and prioritisation of breeding sites, recycling and clean-up campaigns and strong multisectoral cooperation' [2, 7]. A further factor explaining the success of interventions may be the combination with additional interventions, such as 'recycling and clean-up campaigns'.

Specific community characteristics and behaviours, as well as the general cultural system and how it is related to other contributing factors (i.e. public health infrastructure; commitment of the local and national government; and participation of private and public institutions, non-governmental organisations and international stakeholders) have to be taken into account. These elements could have been very influential in the outcome of such community-based programmes, and they could be unique to a given community, country or region. In other contexts, it may not be culturally acceptable to introduce live insects into household water-storage containers, as in Thailand [1].

However, considering the success of the Vietnam-based studies, it would be beneficial to study whether the success can be replicated elsewhere in communities with similar – or perhaps even different -–characteristics.

The community effectiveness studies conducted in Costa Rica, Mexico, and USA showed highly variable results. An intervention control study (6) noted that in Mexico, despite successful community participation, *Mesocyclops longisetus* failed to thrive in high numbers even in containers where water was left undisturbed. The lack of protozoa and algae as supplementary diet for the copepods in the study environment significantly affected the copepod growth rates. A prolonged dry season was also seen as an additional limiting variable in sustaining copepod population. The investigators concluded that community-based vector control programmes, at least in the north-eastern parts of Mexico, may result in some degree of success if the following prerequisite ecological factors are satisfied: (i) rich nutrient environment provided by the breeding sites, (ii) high copepod inoculation rates and (iii) availability of a regional rainfall pattern preventing desiccation of temporary larval sites. This study documented that despite eliciting successful community involvement in copepod programmes, successful reproduction of initial copepod numbers inoculated into larval habitats remains a key point in controlling larval densities.

In other words, not only community participatory factors play a role for a successful introduction of copepods as an intervention in dengue vector control, but also environmental factors.

One of the community effectiveness studies carried out in the USA (8) reported a noticeable time delay for the copepods to eliminate mosquito larvae in treated tyres and attributed it to concerns on reproduction and the significant time it takes to build up the copepod population. The investigator of this study also mentioned that there may be a need to apply supplementary larvicides to kill mosquito larvae already in containers which are too large for the newly inoculated population of copepods.

Other studies, which reported a relative degree of success were insufficient findings for drawing conclusions on whether the use of copepods as single intervention for dengue vector control could be successful and sustained at the same time. Study (7), for example, suffered from not having a well-designed method, specifically in terms of the use of clear and consistent outcome measures, both entomological indices and human epidemiological parameters; lacking a good general study design; poor techniques in administering the intervention; and adequate and appropriate use of statistical tests for data validation.

According to one mixed (efficacy and effectiveness) study conducted in Laos (9), copepods provided control of *Aedes* larvae in wells but not in water-storage containers. Regular cleaning and large turnover of water of these storage containers hindered copepod survival and sustainability. The authors concluded that if copepods were to be integrated in any existing vector control programme, community behavioural practices such as cleaning and maintenance of water-storage containers would need to change.

Similarly, a mixed study in Honduras (11) showed that without precautions, copepods will sometimes disappear from treated containers and require re-inoculation. The authors concluded that a control programme will not succeed if the copepods are simply introduced to containers and then forgotten. It was emphasised that the effectiveness for mosquito control would require constant effort in maintaining container conditions essential for copepod survival.

Further operational aspects, not directly resulting from the systematic review, are discussed in the scientific literature: In many countries, copepods must have approval by local boards for exotic biological control agents. For copepod interventions to be sustainable, high levels of efficacy and capacity for mass production may be necessary, especially in relation to the natural enemy and to environmental conditions (inoculative *vs.* inundative release) [6, 33].

Discussion of limitations

This systematic review included single intervention studies only and therefore might have missed out on works that combined copepods with other dengue vector control agents such as larvivorous fish, *Bacillus thuringiensis var. israelensis* (*Bti*) and Temephos. It excluded non-trial studies, as well as those which focused mainly on the predatory potential of copepods. Moreover, a significant number of relevant studies used non-randomised design, with variable intervention characteristics and outcome parameters. However, as the aim of this study was to investigate community effectiveness of copepods, these limitations had no direct effect on the research question.

Conclusion

In conclusion, the use of copepods as a single intervention may be a community effective and sustainable dengue vector control method to control dengue vectors and dengue transmission. However, this may only be possible provided several specific criteria are met. As clearly shown in the fives studies conducted in Vietnam, these would include rigid delivery of intervention, development of community management committees and collaborators, efficient mobilisation and sustained interest of the community residents. Unfortunately, this evidence is very limited, as in other geographical areas, no such success could be demonstrated. Very few studies are available overall.

Hence, we recommend that further studies be developed with the following considerations: (i) conducting medium- or large-scale and long-term, sustainable studies using communities or households as unit of allocation in other endemic regions; (ii) use of quality research designs, such as cluster randomised control trials supplemented by qualitative methods; (iii) use of clearly defined outcome measures, both entomological indices and human epidemiological parameters; (iv) efficiently administered and documented interventions; and (v) use of appropriate statistical tools of analysis.

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