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Operational deployment of the native cyclopoid copepod Megacyclops viridis for integrated biological control of Aedes albopictus in Germany

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Abstract

Background

The invasive Asian tiger mosquito, *Aedes albopictus*, has become established in an increasing number of German municipalities, causing severe biting nuisance and increasing the risk of local arbovirus transmission. Current control programmes rely primarily on source reduction through community participation and larval control using *Bacillus thuringiensis israelensis* (Bti). However, the limited residual activity of Bti necessitates repeated applications, resulting in considerable operational costs. This study evaluated the integration of Bti with the native cyclopoid copepod *Megacyclops viridis* as a sustainable biological control strategy against *Ae. albopictus*.

Methods

Field-collected *M. viridis* were morphologically identified and evaluated for compatibility with Bti, predation of different mosquito larval instars, persistence in 70-L semi-field containers, and suitability for mass rearing, cold storage, and standardized packaging for operational deployment. In spring 2025, more than 3,500 bags containing approximately 200 copepods each were deployed in rainwater containers and cisterns throughout *Ae. albopictus*-infested districts of Heidelberg. Container indices and ovitrap egg densities were compared with those in the neighbouring municipality of Eppelheim, where routine control measures were implemented without copepod release.

Results

Bti caused no detectable mortality of *M. viridis*, even at concentrations substantially exceeding those used operationally, and copepods reproduced successfully during 35 days of exposure to both 5 and 10 ppm Bti. A single adult female reduced the survival of first-instar *Ae. albopictus* larvae by 82.7% within 24 h but showed little or no predation on later instars. Under semi-field conditions, Abbott-corrected larval mortality reached 92.3–96.7% at the higher copepod density and remained consistently high throughout the 54-day study period. *M. viridis* could be mass-produced, packaged at approximately 200 individuals per bag, and stored at 6 °C for up to 10 weeks without a significant decline in survival. A total of 2,201 copepod bags were deployed to 2,042 breeding sites across 1,062 properties.

Follow-up inspections demonstrated a substantially lower container index in copepod-treated areas (17.5%) than in the untreated reference municipality (57.5%; Fisher's exact test, $P < 0.0001$). In districts with the highest treatment coverage, the container index was reduced to 6.8%. Ovitrap surveillance further showed approximately 50% lower ***Aedes*** egg densities in copepod-treated areas.

Conclusions

The native cyclopoid copepod *Megacyclops viridis*is fully compatible with Bti and can be mass-produced, stored, and operationally deployed under routine control conditions. Its integration into existing Bti-based control programmes provides an effective, environmentally sustainable, and operationally feasible

strategy for long-term suppression of *Ae. albopictus* in urban container habitats. This integrated biological approach has the potential to reduce treatment frequency, lower operational costs, and improve public acceptance of mosquito control programmes.

Background

The invasive Asian tiger mosquito, *Aedes albopictus*, has spread rapidly from its initial foci in south-western Germany to numerous municipalities, including urban areas farther north. Established populations cause severe daytime biting nuisance, impair residents' quality of life, and require sustained municipal control programmes. Moreover, because *Ae. albopictus* is a competent vector of dengue, chikungunya, and Zika viruses, its establishment increases the risk of autochthonous arbovirus transmission following the introduction of viraemic travellers during periods of favourable climatic conditions.

Mosquito control programmes in Germany follow the principles of integrated vector management (IVM), combining surveillance, source reduction, public education, and larval control. In areas with established *Ae. albopictus* populations, intensive door-to-door inspections are conducted to eliminate or treat artificial breeding sites, while community participation is promoted through extensive public information campaigns. Larval control relies primarily on formulations of *Bacillus thuringiensis israelensis* (Bti), a highly selective microbial larvicide with an excellent environmental safety profile.

However, the residual activity of Bti under field conditions is limited because its insecticidal toxins degrade within a relatively short period. Consequently, breeding containers require repeated treatments, typically every three weeks throughout the mosquito season, resulting in substantial personnel requirements and operational costs. Although complementary approaches such as the sterile insect technique can enhance area-wide suppression, the long-term management of permanent water-filled containers remains one of the major operational challenges [1].

Cyclopoid copepods are naturally occurring predators of newly hatched mosquito larvae and are capable of establishing persistent populations in stable aquatic habitats. Numerous laboratory, semi-field, and field studies have demonstrated high predation rates against container-breeding *Aedes* mosquitoes by species of *Megacyclops*, *Mesocyclops*, and *Macrocyclops* [2–14]. Their efficacy depends on several factors, including copepod species and size, mosquito species and developmental stage, habitat characteristics, prey density, and the availability of alternative food resources.

Among the native European species, *Megacyclops viridis* has shown particularly high predatory activity against first-instar larvae of both *Ae. albopictus* and *Ae. japonicus*, making it a promising biological control agent for temperate regions [2, 5].

The strongest evidence for the operational use of cyclopoid copepods originates from community-based dengue control programmes in Vietnam, where the introduction of *Mesocyclops* spp., combined with source reduction and sustained community participation, resulted in the long-term suppression or local

elimination of *Aedes aegypti* in numerous communities [15–17]. Similar integrated approaches have subsequently been evaluated in Thailand, Honduras, and the United States with encouraging results [12–14, 19–22, 24]. Collectively, these studies demonstrate that copepods can provide effective long-term biological control when integrated into comprehensive mosquito management programmes.

Despite these promising findings, the routine large-scale use of cyclopoid copepods against *Ae. albopictus* in Europe remains uncommon. In particular, standardized procedures for mass production, quality assurance, storage, packaging, and operational deployment under European conditions have not been described in sufficient detail.

Our previous laboratory investigations identified the native cyclopoid copepod *Megacyclops viridis* as a highly efficient predator of newly hatched *Ae. albopictus* larvae, providing the biological basis for operational evaluation under field conditions [2].

The present study evaluated the operational implementation of *M. viridis* as a component of an integrated biological control programme against *Ae. albopictus* in Germany. Specifically, we assessed (i) compatibility with Bti, (ii) instar-specific predation, (iii) persistence and efficacy under semi-field conditions, (iv) the feasibility of standardized mass production, storage, and packaging, and (v) operational performance following large-scale deployment in urban container habitats.

Methods

Study design

The study comprised laboratory experiments on Bti compatibility and larval predation, semi-field evaluations in rainwater containers, the development of standardized procedures for mass rearing, packaging, and cold storage of *M. viridis*, and an operational field evaluation conducted in Heidelberg, Germany.

Operational outcomes in Heidelberg were compared with those in the neighbouring municipality of Eppelheim, where routine *Ae. albopictus* control continued without copepod deployment. Because this field evaluation was designed to assess operational feasibility under routine programme conditions, the study followed an observational rather than randomized design.

Collection and identification of native copepods

Copepods were collected in July 2024 from a permanent floodplain pond near Philippsburg, Germany (49°14'44.6"N, 8°26'45.0"E), using a long-handled plankton net (100-µm mesh) swept through submerged littoral vegetation. Water pH and conductivity were measured in situ with a portable multiparameter meter (Combo pH & EC HI98130; Hanna Instruments, USA). Adult cyclopoid females carrying egg sacs were isolated in the field and transported to the laboratory in pond water for subsequent identification and colony establishment.

Species identification was based on adult females using the taxonomic keys of Einsle and of Błędzki and Rybak [28, 29]. Diagnostic structures, including the fourth and fifth swimming legs, terminal thoracic segments, abdomen, and furca, were dissected, mounted in glycerol, and examined using stereo- and compound microscopy.

Compatibility of Megacyclops viridis with Bti

To evaluate whether *M. viridis* can be integrated into existing Bti-based control programmes, potential effects of Bti on copepod survival and reproduction were investigated under laboratory conditions.

Suspensions of VectoBac® WG (potency: 3,000 ITU mg^{-1}) were prepared in filtered tap water to obtain final concentrations of 1, 5, 10, and 50 ppm. Fresh suspensions were mixed immediately before each experiment.

For the acute compatibility assay, three replicate 200-mL beakers containing five adult female *M. viridis* each were prepared for every Bti concentration (1, 10, and 50 ppm). Corresponding tap-water controls were included. Identical experimental conditions were used with second-instar *Cx. pipiens* s.l. larvae to verify Bti activity. Survival was recorded after 48 h.

To assess long-term effects, five replicate containers containing five adult female copepods each were maintained in either tap water or Bti suspensions (5 or 10 ppm) for 35 days without supplementary food. Containers were kept at 22 ± 2°C under an 18:6 h light:dark photoperiod. Evaporation losses were replenished weekly using the corresponding treatment solution. At the end of the experiment, surviving adults and late-stage copepodids were counted.

Instar-specific predation experiments using larval instars of Aedes albopictus and Culex pipiens s.l.

Predation on the first three larval instars of *Ae. albopictus* and *Cx. pipiens* s.l. was assessed using designs adapted from Chansang et al. and Rey et al. [14, 25]. For each mosquito species and instar, five replicate 500-mL containers received one adult female *M. viridis* and five larvae of the respective species and instar. Corresponding containers without copepods served as controls. *Ae. albopictus* larvae originated from a laboratory colony; freshly hatched larvae were used immediately for the first-instar assay and synchronized cohorts were reared to the second or third instar. *Cx. pipiens* s.l. larvae were obtained from field-collected egg rafts. A small quantity of powdered fish food (Tetra TabiMin) was added to each container. Containers were held at 22 ± 2°C under a 16:8-h light:dark photoperiod. Surviving larvae and identifiable larval remains were counted after 24 h.

Semi-field evaluation in rainwater containers

Semi-field experiments were conducted in shaded outdoor conditions in Heidelberg, Germany, using 70-L plastic rainwater containers filled with tap water. To promote the development of natural microbial communities and algal growth, containers were conditioned outdoors for two weeks before the

experiments. All containers were covered with fine mesh to prevent natural colonization by mosquitoes. Freshly hatched **Aedes albopictus** larvae (≤ 3 h old) were obtained from the laboratory colony.

Three experimental treatments were established. Group A consisted of three containers inoculated with 60 **M. viridis** and challenged with 300 first-instar larvae at each introduction. Groups B and C each received 120 copepods per container and were challenged with either 600 or 240 larvae, respectively. Three untreated containers receiving 600 larvae served as controls.

Mosquito larvae were introduced at the start of the experiment and subsequently at 9-day intervals. Six days after each introduction, all remaining larvae and pupae were collected using a plankton net and counted. Copepods that were inadvertently collected were immediately returned to their original container. Separate nets and pipettes were used for each treatment to prevent cross-contamination. Three days later, a new cohort of first-instar larvae was introduced.

After 54 days, the contents of each container were filtered through a 150-µm mesh, and surviving adult copepods and late-stage copepodids were counted under a stereomicroscope.

Mass rearing, harvesting, packaging and storage of copepods

To enable routine operational application, a standardized mass-rearing system for **M. viridis** was established considering standardized water chemistry, temperature, photoperiod as well as food quality and quantity. Stock cultures were founded from individually isolated egg-bearing females collected from natural populations. Following morphological confirmation of species identity, offspring from multiple females were pooled to maintain genetic diversity.

Mass cultures were maintained in ten 200-L containers at 22 ± 2°C under a 16:8 h light:dark photoperiod. Reverse-osmosis water was remineralized to approximately pH 7.2, conductivity 1,000 µS cm^{-1}, total hardness 14 °dGH, and carbonate hardness 8 °dKH. Cultures were fed every three days with laboratory cultures of **Paramecium caudatum** and **Chlorella** sp.

Adults and late-stage copepodids were harvested after approximately three weeks using a 250-µm plankton net, concentrated in remineralized water, and packaged into 250-mL plastic bags using a tailor-made packaging system to obtain approximately 200 adult copepods and late-stage copepodids. Bags were labelled and stored at 6°C until field deployment. To maintain culture quality, approximately 20 L of water were replaced after every third harvest.

Quality control and cold storage

For quality control, three bags were randomly selected from each production batch. Copepods were recovered using a 250-µm sieve, transferred to a white tray, and counted under a stereomicroscope. Mean numbers of adults and late-stage copepodids per bag were calculated.

Cold-storage performance was evaluated using bags containing approximately 100 copepods. Three bags were analysed immediately after packaging to determine baseline density, whereas the remaining bags were stored at 6 ± 1°C (Gastro Hero, model GNH1410 TNM). At two-week intervals for up to ten weeks, three bags were removed from storage and analysed for the number of surviving adult copepods and late-stage copepodids.

Operational deployment in Heidelberg

During the 2024 preparatory phase, trained field staff repeatedly visited properties in *Aedes albopictus*-infested districts of Heidelberg to identify and treat potential breeding sites with *Bacillus thuringiensis israelensis* (Bti). Permanent water-holding containers, particularly rainwater barrels and cisterns, were georeferenced and recorded using the QField/QGIS platform to establish a deployment database for the 2025 field season.

Between April and mid-May 2025, all previously mapped properties were revisited for copepod deployment. Prior to release, copepod bags were acclimatized to ambient temperature for approximately 1 h, protected from direct sunlight during transport, and used within four weeks of packaging. Containers with a capacity of up to 200 L received one bag containing approximately 200 adult or late-stage copepodid *M. viridis*, whereas larger containers received additional bags in proportion to their volume. Immediately before copepod release, each container was treated with one Bti tablet (Culinex® Tab Plus; 1,000 ITU mg^{-1}) per 50 L of water to eliminate existing mosquito larvae while allowing subsequent control of newly hatched larvae by the copepods.

For each treated container, the release date, geographic location, container type, and number of bags applied were documented. Residents received verbal and written information explaining the purpose of the treatment and were asked not to empty inoculated containers completely. If household members were absent during field visits, copepod bags could be collected from a municipal information centre for self-application.

Operational entomological evaluation

Approximately 2.5 months after copepod deployment, during the second half of July 2025, entomological surveys were conducted in the treated districts of Pfaffengrund, Ochsenkopf, and Kirchheim. A total of 97 rainwater containers were inspected. Each container was sampled by sweeping a 250-µm plankton net twice for 1 min in a figure-eight pattern extending from the water surface to the bottom. The presence of mosquito larvae and copepods was recorded. Mosquito larvae were initially classified morphologically as *Cx. pipiens* s.l. or *Aedes* spp. Suspected *Ae. albopictus* larvae were transported to the laboratory for confirmation using standard morphological identification keys [30]. After examination, copepods were returned to their original containers.

During the same survey period, 73 rainwater containers were examined in the neighbouring municipality of Eppelheim, where routine source reduction and Bti applications were performed but copepods had not been released. For both study areas, the container index (CI) was calculated as the percentage of inspected containers containing *Ae. albopictus* larvae.

To complement larval surveys, ovitrap monitoring was performed using black plastic containers filled with 1.5 L of tap water and equipped with a wooden oviposition paddle (200 × 25 × 4 mm). Sixteen ovitraps were deployed in Pfaffengrund between 29 July and 25 August 2025, whereas 26 traps were deployed in Eppelheim between 6 and 26 August 2025. Traps were arranged on an approximately 250 × 250 m sampling grid. Eggs were counted under a stereomicroscope and identified based on size, morphology, and exochorion characteristics. A subset of eggs was flooded and reared to the third larval instar to confirm species identity. Because monitoring periods differed between study areas, oviposition activity was standardized as the number of eggs per trap-day.

Statistical analysis

Descriptive data are presented as counts, percentages, means ± standard deviation (SD), or means ± standard error (SE), as appropriate. Larval mortality in the semi-field experiments was corrected for natural mortality using Abbott's formula [27]. Treatment effects in the semi-field experiments were analysed using linear mixed-effects models, whereas copepod population development was evaluated by one-way analysis of variance (ANOVA). Laboratory bioassays and ovitrap data were compared using two-sample *t*-tests or Welch's *t*-tests, depending on variance homogeneity.

For the operational container comparison, two-sided Fisher’s exact tests were recalculated from the reported counts. Wilson 95% confidence intervals (CIs) were calculated for container indices. A 3 × 2 chi-square test was used to compare container positivity among the high-coverage areas of Heidelberg, Kirchheim, and Eppelheim. Pairwise comparisons were performed using Fisher’s exact tests with Bonferroni correction for multiple testing. All analyses were conducted in R version 4.4 or later. Unless otherwise specified, all statistical tests were two-sided, and $P < 0.05$ was considered statistically significant

Results

Collection and identification of Megacyclops viridis

The source pond had a pH of 7.2–7.6, a conductivity of 400–800 µS cm^{-1}, a total hardness of 13 °dH, and a carbonate hardness of 12 °dH. All collected adult cyclopoid females were morphologically identified as *Megacyclops viridis*. Diagnostic characteristics included a two-segmented fifth swimming leg with a laterally expanded proximal segment, a short inner apical spine on the distal segment that did not extend beyond the segment margin, and a sixth furcal seta longer than the furcal ramus.

Compatibility of Megacyclops viridis with Bti

No mortality or behavioural impairment of adult **M. viridis** was observed during 48 h of exposure to Bti concentrations of 1, 10, or 50 ppm. In contrast, all **Cx. pipiens** s.l. larvae exposed to these concentrations died within 48 h, whereas all control larvae survived.

After 35 days without supplementary food, no copepods remained alive in the tap-water controls. By contrast, mean numbers of 9.0 ± 1.4 and 11.0 ± 1.6 adult and late-stage copepods were recorded in the 5- and 10-ppm Bti treatments, respectively (Fig. 1). No significant difference was detected between the two Bti concentrations.

Predation experiments

A single adult female **M. viridis** reduced survival of first-instar **Ae. albopictus** larvae by 82.7% within 24 h, whereas survival in the control group reached 98.6% (Fig. 2a). No significant predation was observed against second- or third-instar larvae.

Predation on first-instar **Cx. pipiens** s.l. larvae was substantially lower (28.6%), and no significant effects were detected for later larval instars (Fig. 2b).

Semi-field efficacy and copepod population development

Control mortality averaged 29.1 ± 18.9%. After Abbott correction, mean larval mortality reached 73.3 ± 22.1% in Group A, 92.1 ± 10.9% in Group B, and 97.2 ± 5.9% in Group C (Fig. 3). Larval mortality remained consistently above 92% throughout the study period in Groups B and C.

Linear mixed-effects analysis revealed a highly significant treatment effect (**P** < 0.001). Pairwise comparisons with Holm correction showed that all copepod treatments differed significantly from the untreated control. Mortality in Groups B and C was significantly higher than in Group A, whereas no significant difference was detected between Groups B and C (**P** = 0.280).

Population development

Copepod populations increased in all inoculated containers. Final abundance was reported to be approximately 22-fold the initial number in group A, sixfold in group B and 26-fold in group C, indicating reproduction and persistence under semi-field conditions (Fig. 4).

Mass production

All ten production cultures yielded harvestable copepod populations after approximately three weeks. Each production cycle generated approximately 600 bags. Mean package contents were 193.7 ± 48.9, 231.7 ± 35.1, and 206.0 ± 18.0 copepods in the three production runs, corresponding to an overall mean of 210.4 ± 19.4 individuals per bag. Package-to-package variation decreased following optimization of the concentration procedure.

Cold storage

No significant decline in copepod survival was detected over the 10-week storage period (ANOVA, P = 0.123), indicating that cold storage at 6°C is suitable for operational stock management.

Operational field deployment

Mapping identified at least one permanent water-holding container or cistern at 1,291 properties within the *Ae. albopictus*-infested districts of Heidelberg. Between April and mid-May 2025, four trained field staff visited 1,062 properties (82.3%) and inoculated 2,042 breeding containers with a total of 2,201 bags containing *Megacyclops viridis*. In addition, 1,339 copepod bags were distributed through the municipal information centre for self-application by residents who were unavailable during field visits. On average, each property visit, including resident consultation and documentation, required approximately 10 min.

Operational efficacy of copepod deployment

Container index: *Ae. albopictus* larvae were detected in 17 of 97 inspected containers in the copepod-treated districts, corresponding to a container index (CI) of 17.5% (95% CI: 11.2–26.3%). In contrast, 42 of 73 containers were positive in the neighbouring municipality of Eppelheim, where routine source reduction and Bti treatments were implemented without copepod release, resulting in a CI of 57.5% (95% CI: 46.1–68.2%). The odds of detecting *Ae. albopictus* larvae were significantly lower in the copepod-treated districts (odds ratio = 0.16, 95% CI: 0.08–0.32; Fisher's exact test, $P < 0.0001$; Fig. 5).

Within Heidelberg, treatment efficacy varied among districts. In Ochsenkopf and Pfaffengrund, where copepod deployment coverage was highest (> 82%), only four of 59 inspected containers were positive for *Ae. albopictus* larvae (6.8%; 95% CI: 2.7–16.2%). In Kirchheim, where deployment coverage was lower, 13 of 38 containers were positive (34.2%; 95% CI: 21.2–50.1%). Container positivity differed significantly among the three study areas ($\chi^2 = 37.10$, df = 2, $P < 0.0001$; Fig. 6). Post hoc comparisons with Bonferroni correction showed that both high-coverage districts differed significantly from Kirchheim and Eppelheim, whereas the difference between Kirchheim and Eppelheim was not statistically significant.

Copepods were recovered from 41 of the 97 inspected containers (42.3%). Of these, 39 containers (95.1%) were free of *Ae. albopictus* larvae. In contrast, larvae were detected in 15 of the 56 containers in which copepods were not recovered. This association between copepod presence and the absence of *Ae. albopictus* larvae was statistically significant (Fisher's exact test, $P = 0.006$). During interviews, residents most frequently reported complete emptying of rainwater containers as the reason for copepod loss.

Ovitrap monitoring

A total of 7,708 *Ae. albopictus* eggs were collected from 26 ovitraps deployed in the untreated municipality of Eppelheim between 6 and 26 August 2025, corresponding to a mean of 296.5 eggs per

trap. In the copepod-treated district of Pfaffengrund, 3,032 eggs were collected from 16 ovitraps deployed between 29 July and 25 August 2025, corresponding to a mean of 189.5 eggs per trap.

To account for the different deployment periods, oviposition was standardized as eggs per trap-day. Mean egg density was 14.82 eggs per trap-day in Eppelheim and 7.02 eggs per trap-day in Pfaffengrund, representing a 52.6% reduction in oviposition activity in the copepod-treated area (Fig. 7). This difference was statistically significant (Welch's *t*-test, $P = 0.002$).

Discussion

This study demonstrates that the native cyclopoid copepod *Megacyclops viridis* can be successfully integrated into routine *Aedes albopictus* control programmes in Germany. The combined use of *M. viridis* and *Bacillus thuringiensis israelensis* (Bti) provides a complementary biological control strategy that couples the rapid elimination of existing mosquito larvae with long-term suppression of newly hatched larvae through sustained copepod predation. In addition to demonstrating biological efficacy, this study establishes standardized procedures for the mass production, quality control, storage, packaging, and operational deployment of *M. viridis* under routine programme conditions.

The complementary modes of action of Bti and *M. viridis* explain the high and sustained efficacy observed under operational conditions. Whereas Bti rapidly eliminates existing mosquito larvae, copepods establish persistent populations within suitable breeding containers and prey continuously on newly hatched first-instar larvae. This combination extends the effective control period beyond that achievable with Bti alone while maintaining the exclusive use of biological control agents.

Consistent with previous studies, predation was largely restricted to first-instar *Ae. albopictus* larvae and was substantially lower for *Cx. pipiens* s.l. larvae. This difference probably reflects contrasting larval behaviour. Whereas newly hatched *Aedes* larvae frequently occupy deeper water layers where copepods forage, *Culex* larvae remain predominantly at the water surface. In addition, the dense body setae of *Culex* larvae may reduce capture success, as previously suggested by Marten and colleagues [22].

Our findings are consistent with previous operational studies demonstrating the potential of cyclopoid copepods for long-term mosquito control. Community-based programmes in Vietnam remain the best-known example, where *Mesocyclops* spp., combined with source reduction and public participation, resulted in sustained suppression of *Aedes aegypti* over many years [15–18]. Similar integrated approaches have subsequently been reported from Thailand, Honduras, and the United States [3, 10–12, 14, 19, 20, 21, 24]. Collectively, these studies support the concept that copepods can provide durable biological control when integrated into comprehensive mosquito management programmes.

In contrast to previous studies, the present work focuses on operational implementation under Central European conditions and provides detailed information on standardized mass production, quality assurance, cold storage, packaging, and large-scale field deployment of an indigenous copepod species.

These operational aspects are essential prerequisites for routine implementation but have received comparatively little attention in the published literature.

Complete compatibility between Bti and *M. viridis* represents a key prerequisite for integrated biological control. Even at concentrations substantially exceeding operational application rates, Bti neither affected copepod survival nor impaired reproduction. These findings agree with previous reports and support the continued use of Bti in combination with copepod-based control programmes [12–14, 17–20, 24, 32, 33]

A major contribution of this study is the development of a standardized production and logistics system suitable for routine mosquito control programmes [31]. Reliable mass production, quality-controlled packaging, and cold storage at 6°C to avoid cannibalism and reduce metabolic demand for up to ten weeks make preseason production and flexible operational deployment feasible. These advances address one of the principal barriers that has limited the broader implementation of copepod-based mosquito control in Europe.

The operational field evaluation further supports the effectiveness of this integrated approach. Two and a half months after deployment, the container index in treated districts was approximately one-third of that recorded in the neighbouring reference municipality. The strongest reductions were observed in districts with the highest treatment coverage, emphasizing the importance of comprehensive implementation.

Although copepods were detected in only 42% of inspected containers, their presence was strongly associated with the absence of *Ae. albopictus* larvae. Resident interviews indicated that complete emptying of rainwater containers represented the principal cause of copepod loss. These observations underline the importance of continued public engagement as an integral component of integrated vector management.

This study has several limitations. The field evaluation was not randomized and compared only two neighbouring municipalities. Nevertheless, both municipalities are located within the same metropolitan area and have comparable urban structures, climatic conditions, and vector control programmes (difference is the copepod release), thereby improving the validity of the comparison. Ovitrap monitoring periods differed slightly between study areas; however, oviposition activity was standardized as eggs per trap-day to account for differences in sampling duration. In addition, control mortality in the semi-field experiments was relatively high and variable, reflecting the challenging environmental conditions under semi-field settings. Finally, neither a formal cost-effectiveness analysis nor a quantitative assessment of public acceptance was performed. Future studies should include cluster-randomized or stepped-wedge trial designs across multiple municipalities, together with standardized baseline and follow-up surveillance, to further strengthen the evidence for operational effectiveness. Economic evaluations and assessments of public acceptance would also support the broader implementation of copepod-based mosquito control programmes.

Despite these limitations, this study provides, to our knowledge, the first comprehensive operational framework for the large-scale production, quality-controlled storage, and field deployment of the indigenous cyclopoid copepod *Megacyclops viridis* for the biological control of *Aedes albopictus* in Central Europe. The results demonstrate that *M. viridis* can be successfully integrated with *Bacillus thuringiensis israelensis* (Bti) within an integrated vector management programme, combining the rapid elimination of existing larvae with sustained suppression of newly hatched larvae. With adequate operational coverage and continued community participation, this integrated biological approach has the potential to become a sustainable component of urban *Ae. albopictus* control programmes in temperate regions.

Conclusions

This study demonstrates that the native cyclopoid copepod *Megacyclops viridis* can be successfully mass-produced, quality-controlled, stored, and operationally deployed under routine mosquito control conditions. Integrated with *Bacillus thuringiensis israelensis* (Bti), *M. viridis* provides a complementary biological control strategy that combines the rapid elimination of existing mosquito larvae with sustained suppression of newly hatched *Aedes albopictus* larvae. With high operational coverage and continued community participation, this integrated approach has the potential to become an effective and sustainable component of urban *Ae. albopictus* management in Central Europe. In addition to its biological effectiveness, the operational framework presented here may improve the efficiency, cost-effectiveness, and public acceptance of integrated mosquito control programmes.

Abbreviations

Bti
Bacillus thuringiensis israelensis
CI
confidence interval or container index, as specified
GIS
geographic information system
ITU
international toxic units
SD
standard deviation
SE
standard error.

Declarations

Ethics approval and consent to participate

Not applicable. The study involved mosquito larvae, copepods and routine entomological surveillance and did not enroll human participants or use vertebrate animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

NB conceived and supervised the study and drafted the manuscript with JSC. MT, KW, IP, SS, AT and FD contributed to laboratory, production or field activities and data acquisition. NB and JSC interpreted the data and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

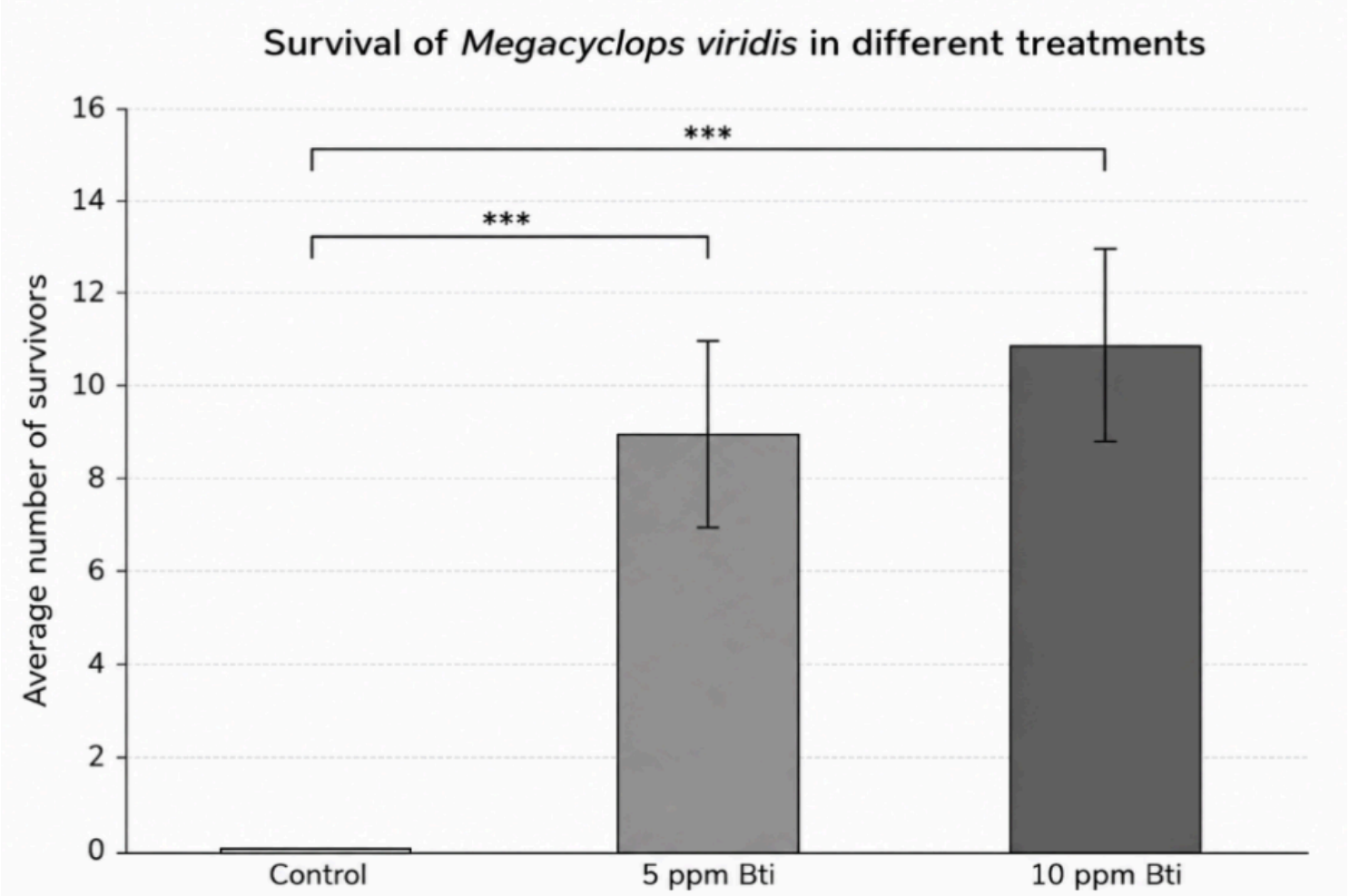


Figure 1

Population development of *Megacyclops viridis*after 35 days in tap water or suspensions containing 5 or 10 ppm *Bacillus thuringiensis israelensis*. Bars show mean ± SD of five replicate containers.

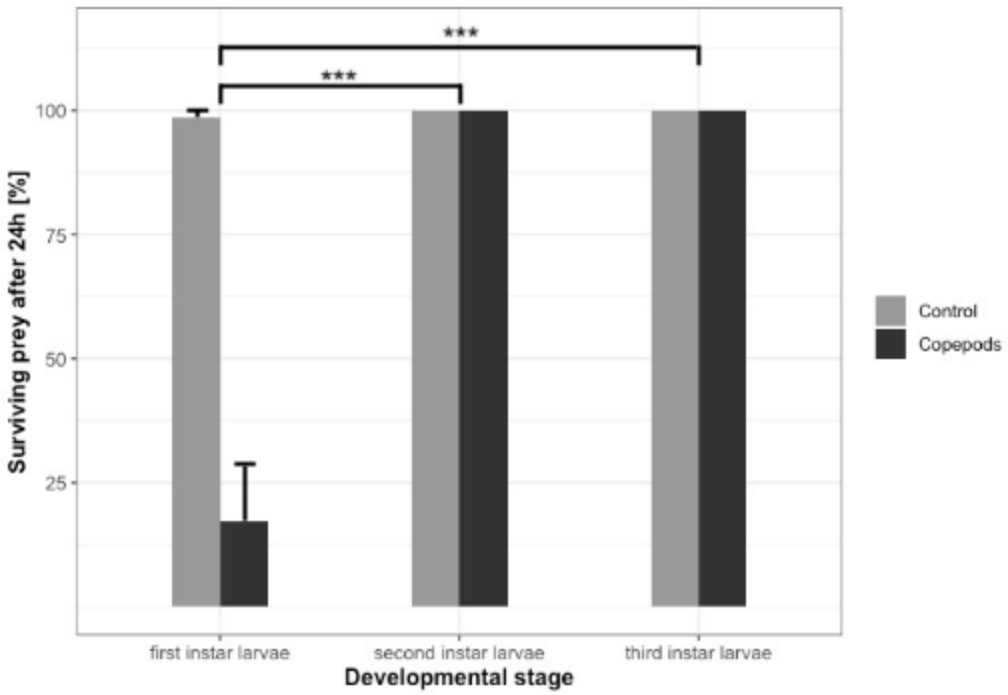


Figure 2

Instar-specific predation by *Megacyclops viridis* during 24-h exposure. (a) *Aedes albopictus* and (b) *Culex pipiens*sensu lato. Each replicate contained one adult female copepod and five mosquito larvae; no-copepod vessels served as controls. Bars show mean survival ± SD (n = 5).

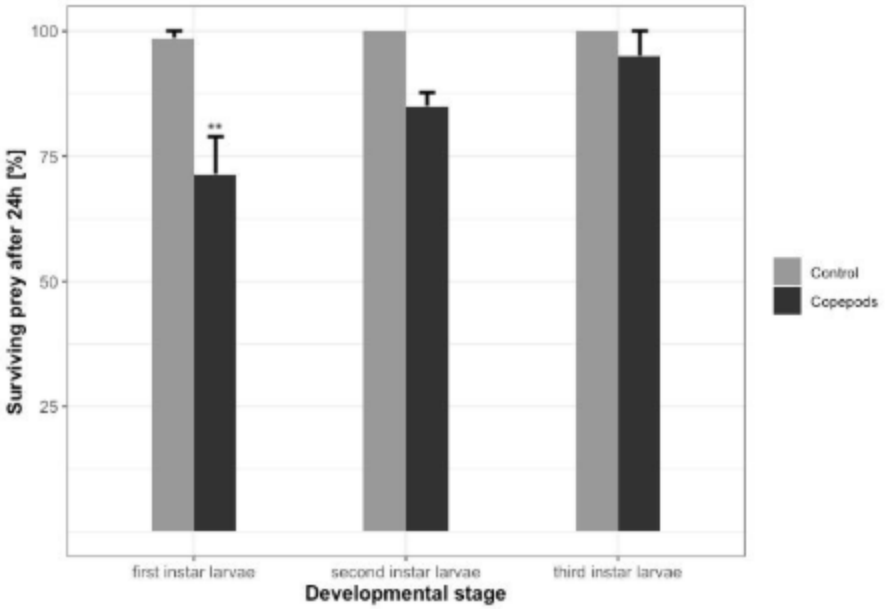


Figure 3

Aedes albopictus mortality during repeated semi-field challenges in 70-L rainwater containers inoculated with different densities of *Megacyclops viridis*in a time interval of 9 days. Group A: 60 copepods and challenge of 300 larvae; group B: 120 copepods and a challenge of 600 larvae; group C: 120 copepods

and a challenge of 240 larvae. Mortality in copepod treatments was corrected using Abbott's formula. Lines or bars show the mean across containers.

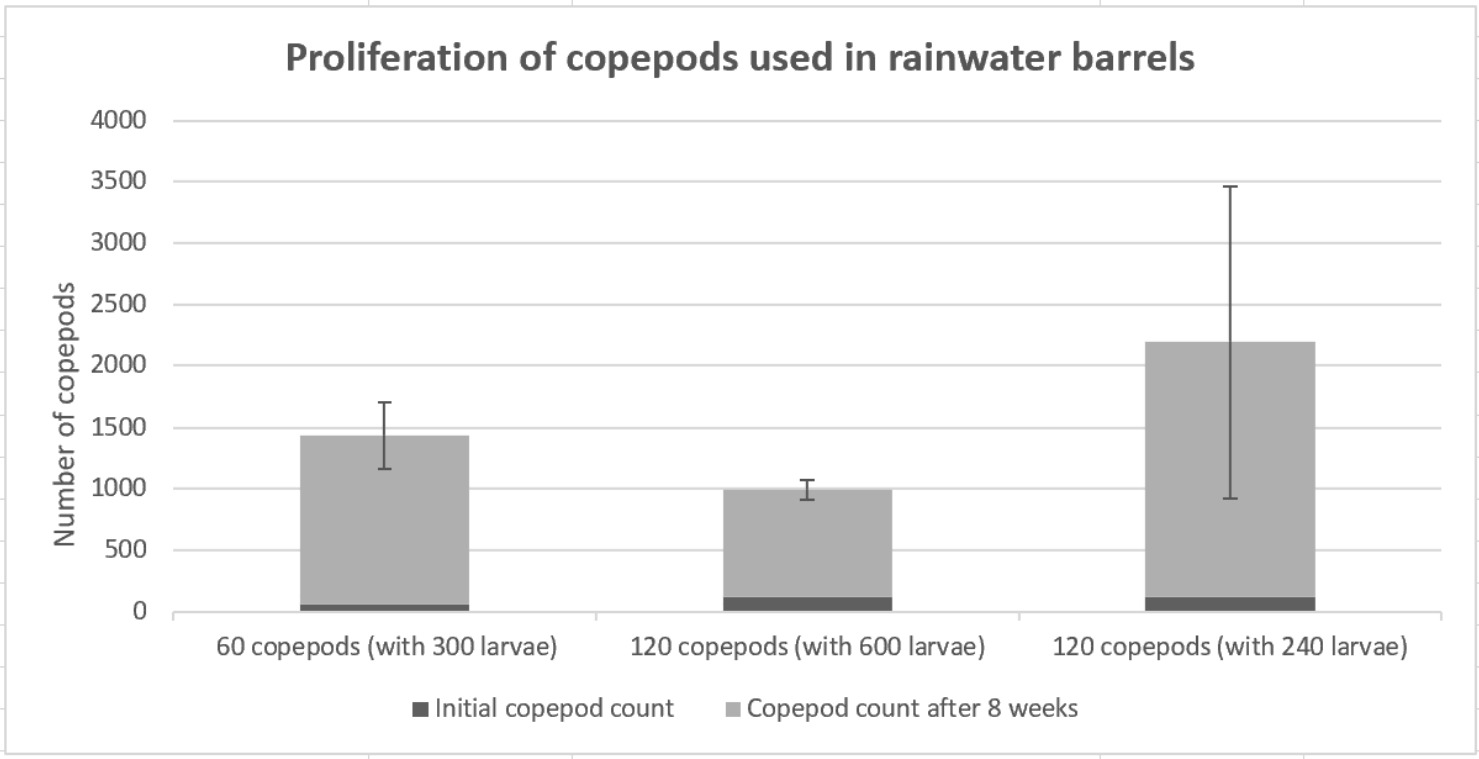


Figure 4

Fold-change in *Megacyclops viridis*abundance in semi-field rainwater containers between inoculation and day 54. Error bars show SD.

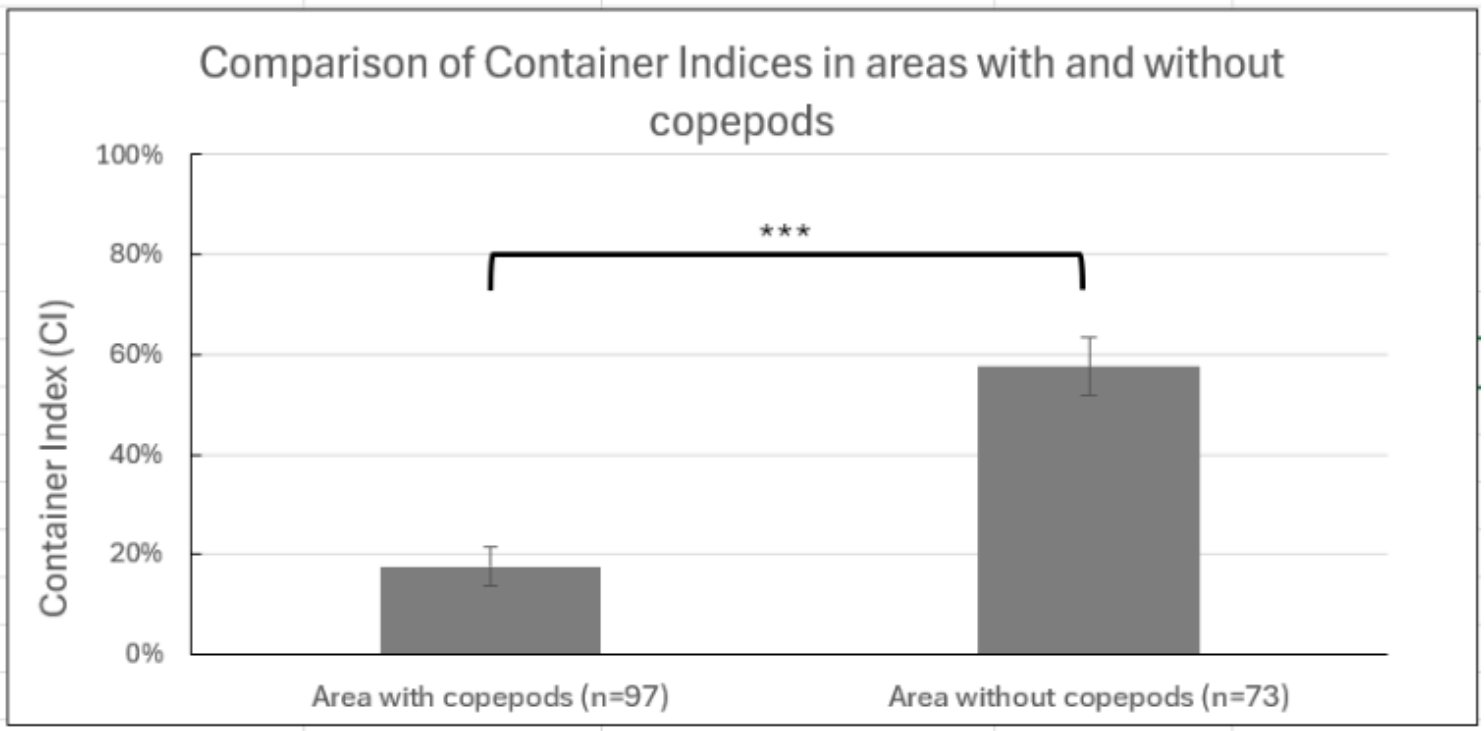


Figure 5

Aedes albopictus container index in copepod-treated Heidelberg districts and neighbouring Eppelheim without copepod release. Values are positive containers divided by inspected containers; error bars represent 95% binomial confidence intervals.

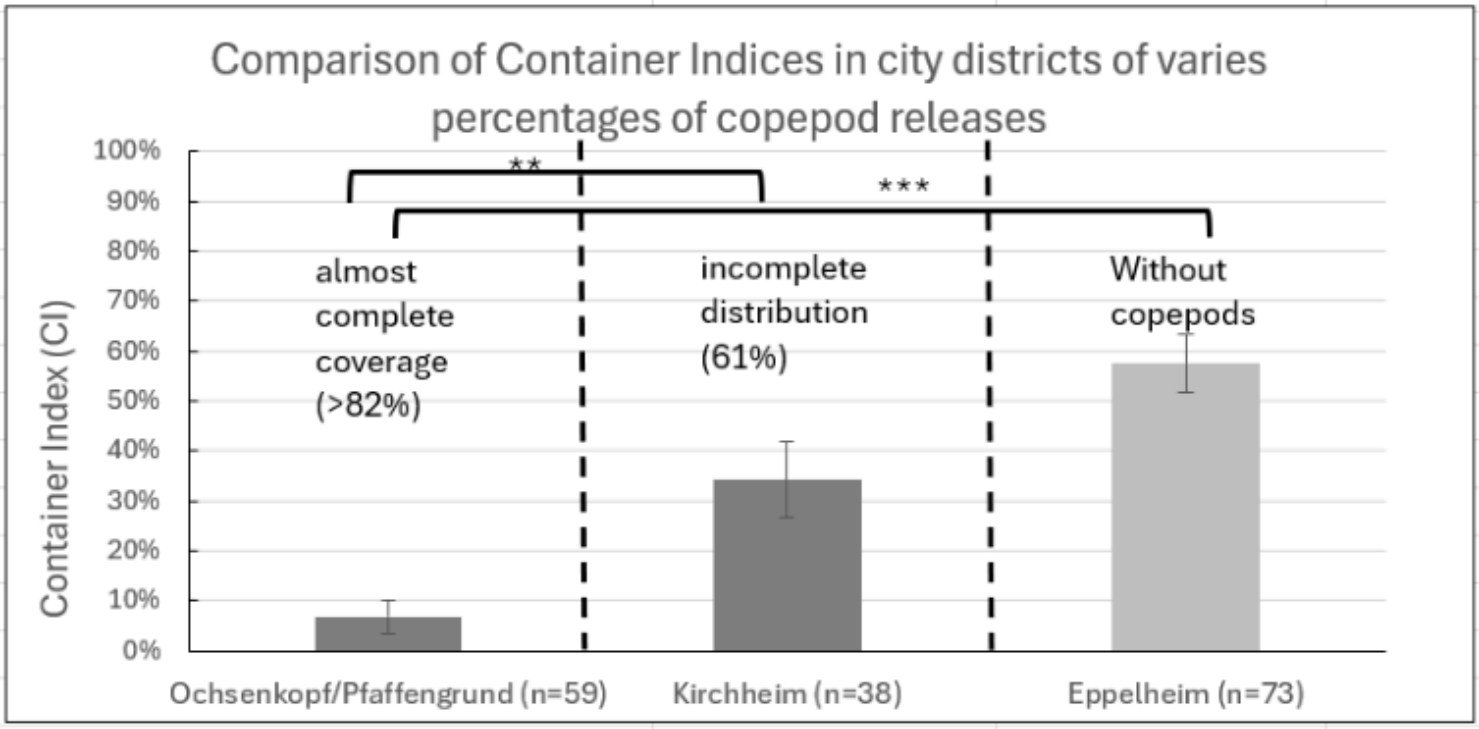


Figure 6

Aedes albopictus container index in high-coverage Heidelberg districts (Ochsenkopf/Pfaffengrund), lower-coverage Kirchheim and Eppelheim. Values are positive containers divided by inspected containers; error bars represent 95% binomial confidence intervals.

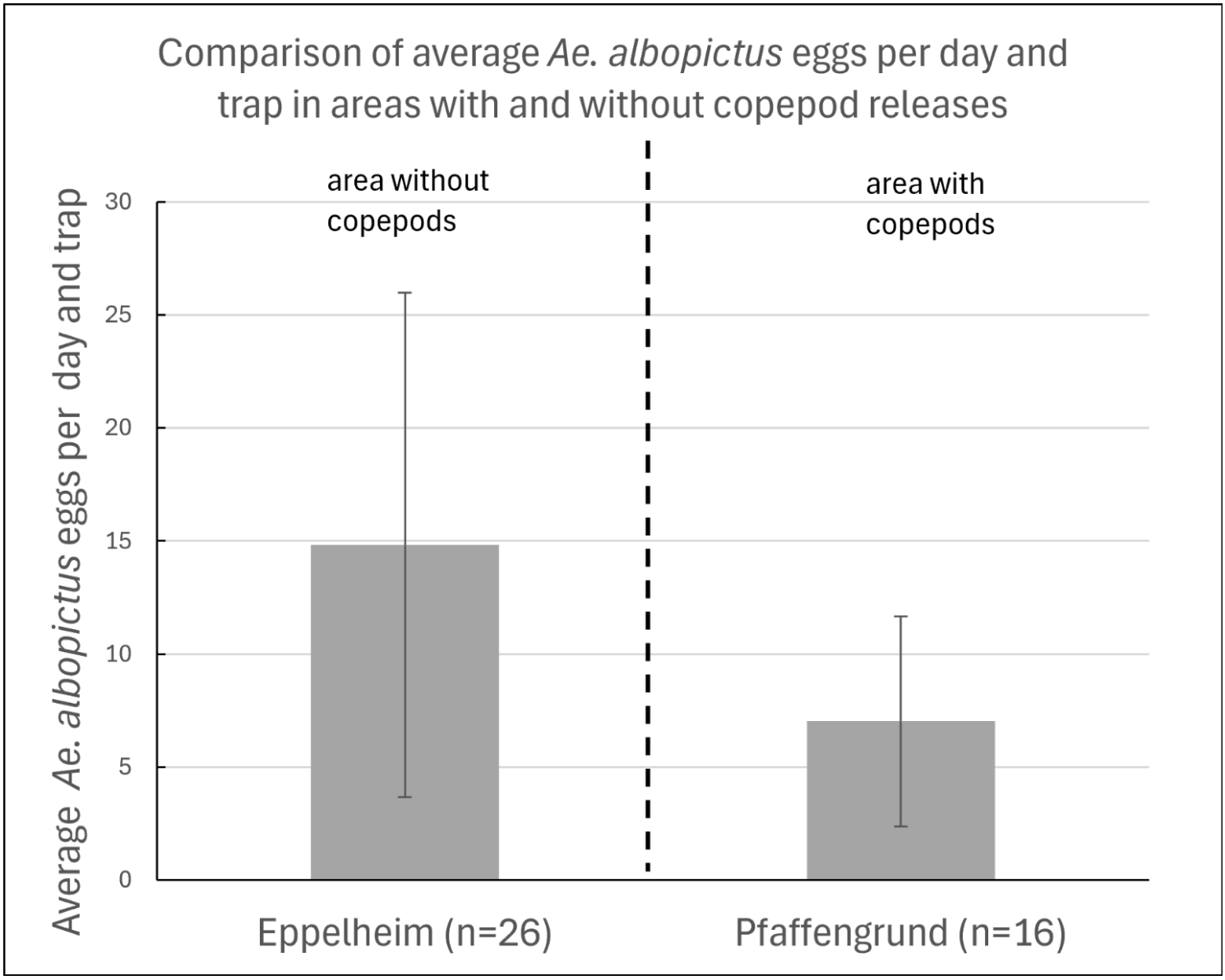


Figure 7

Aedes albopictus egg density in ovitraps in copepod-treated Pfaffengrund and Eppelheim without copepod release, standardized as eggs per trap-day.