Евразиатский энтомол. журнал 24(4): 179–188
doi 10.15298/euroasentj.24.04.01

Methods for collection and evaluation of blood-sucking mosquitoes (Diptera: Culicidae). Preimaginal phases

Методы сбора и оценки кровососущих комаров (Diptera: Culicidae). Преимагинальные фазы

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Key words: Larvae of blood-sucking mosquitoes, methods of quantitative collection, count, sample, sampler, net-frame.

Ключевые слова: личинки кровососущих комаров, методы количественного сбора, подсчёт, проба, пробоотборник, сачок-рамка.

Abstract. There is a lot of information on the number of blood-sucking mosquitoes obtained by different methods and scientists. The use of different methods makes it difficult to compare the data. So, there is a need to unify the existing mosquito counting methods. The practical significance of the Culicidae family representatives makes it highly important to standardise mosquito counts at different development stages. The article provides an overview and a comparative analysis of the currently existing quantitative collection methods of the preimaginal phases of mosquitoes. The authors discuss the advantages and application limitations of different methods. They propose a new method for the quantitative collection of preimaginal stages of the Culicidae mosquitoes in deep water-bodies using the improved version of the Sazonova net-frame.

Резюме. Большое разнообразие сведений по численности кровососущих комаров, полученных разными методами и исследователями, затрудняют сравнительный анализ данных и обусловливают необходимость унификации методов учёта. Практическая значимость представителей семейства Culicidae определяет необходимость проведения стандартизированных учётов на разных стадиях развития комаров. В первом сообщении приводится обзор и сравнительный анализ существующих в настоящее время методов количественного сбора преимагинальных фаз кровососущих комаров. Обсуждаются преимущества и ограничения в применении различных методов. Предложен новый прибор для количественного сбора преимагинальных стадий комаров семейства Culicidae в глубоких водоёмах, разработанный на основании конструкции «сачка-рамки Сазоновой».

Introduction

Mosquitoes are a numerous group of blood-sucking insects in many regions of Russia, especially in the forest-tundra and taiga zones. The practical role of mosquitoes in natural biocenoses and for human's health is great.

In spite of the negative impact of mosquitoes on the economy and human's health, the role of these insects in ecosystems is very important. Mosquitoes, as amphibionts, transfer the organic matter from water-bodies to land, thus improving the soil fertility [Sazonova, 1984]. The positive role of amphibiont twoflies in maintaining the links between terrestrial and aquatic ecosystems is well known [Narchuk, 2003]. In addition, they participate in the creation of an immune layer of the population during the circulation of pathogens of natural focal diseases [Detinova, Smelova, 1973], and also indirectly pollinate flowering plants, feeding on their nectar [Sazonova, 1984]. The role of adult blood-sucking mosquitoes as keepers and carriers of pathogens of many natural focal bacterial and viral diseases of humans and animals, such as tularemia [Gutsevich et al., 1970], West Nile fever [Ponotova et al., 2006; Roslavtseva, 2006; Konovova et al., 2007; Platonova et al., 2007; Aleksejchik et al., 2019] and others is known. At the egg, larval and pupal stages, mosquitoes are safe for humans and play only a positive role in aquatic food chains. Transmission of pathogens occurs only if female mosquitoes feed on warm-blooded animals in foci of infections.

In case of a sharp increase in the relative abundance of popular mosquito species, caused by weather or other environmental conditions, the formation of foci of infectious and arboviral diseases is possible. In this regard, it is very important to monitor and truly evaluate the seasonal and inter-seasonal population dynamics of epidemiologically dangerous mosquito

How to cite this article: Mirzaeva A.G., Panyukova E.V. 2025. Methods for collection and evaluation of blood-sucking mosquitoes (Diptera: Culicidae). Preimaginal phases // Euroasian Entomological Journal. Vol.24. No.4. P.179–188. https://doi.org/10.15298/euroasentj.24.04.01

species in areas with possible formation of natural foci, especially in areas where migratory birds accumulate and there is a possibility of contact with arboviruses being not endemic to a particular region. The need for the continuous monitoring of the seasonal dynamics of mosquitoes increases not only due to the discovery of potential or apparent carriers of natural focal diseases, but also due to the gradual increase in number and enlarging the activity period of mosquitoes of polycyclic species because of the climate warming [Kalmin, 2006; Kilochitsky et al., 2006; Mirzaeva et al., 2007; Mirzaeva, 2008]. Changes in the structure of dominant mosquito species, changes in terms of phenological periods to earlier spring dates, and the possibility of population peaks of polycyclic mosquitoes require a more careful choice of counting methods, as well as trigger the development of new modern methods for collecting and evaluating the number of mosquitoes at different development phases.

Mosquito larvae and pupae at their breeding sites are collected using a variety of tools for manual sampling or semi-automatic samplers. The more diverse the collection methods and the richer the collection sites as habitats and biotopes are, the more complete the species composition and biotopic distribution of mosquito species of the Culicidae family will be described.

Native researchers usually apply the Monchadsky methods for collecting preimaginal mosquito stages [Monchadsky, Radzilovskaya, 1947; Monchadsky, 1952; Gutsevich et al., 1970] but in many cases they refuse from them. This situation is produces by various reasons, including attempts to improve the methods of counting, to adapt them to the specific conditions of the region. In spite of the efforts of a number of native researchers to unify the existing methods of mosquito counting [Detinova et al., 1978; Khalin et al., 2021], there are still no generally accepted methods for quantitative collection of mosquitoes, which would be taken as the only correct and true methods. The method of larval collection with the biocenometer that is actively recommended for use in the works of Nikolaeva [Nikolaeva, 1978, 1980, 1992] and meets international standards, does not satisfy many researchers because of high labour and time costs. This method is criticised by practitioners who believe that it can solve only academic not practical problems. To record the population of mosquitoes when organising measures to regulate the number of these insects, we need an express method to determine the size of the preimaginal mosquito population. Faced with the practical task to decrease the population of mosquitoes, the authors of this review tend to listen to the opinion of practitioners, though some counting methods used by them are quite limited. Moreover, even statistically true counting methods, which give correct results in one area, may not actually work in other areas. This is one problem associated with the unification of the existing methods.

The present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:58516A2F-3D0A-4889-A223-B845D51D5390

Quantitative collection and counting of mosquito eggs

Mosquitoes lay eggs in a wide variety of places where their larvae and pupae breed and grow — in small puddles, large wetlands, shallow waters along river banks, on aquatic plants, in tree and stump hollows, in plant cavities, in accumulation of detritus, in rock splits, in moist soil near rivers and streams, in old-fallen moist leaves, as well as in various artificial containers filled up with water or moist soil material.

In national practice, preimaginal phases of mosquitoes are counted mainly by determining the number of larvae and pupae per unit area of a water-body, *i.e.* their density. Determining the absolute number of mosquitoes in a study area requires not only identifying the density of larvae and pupae and the number of adult mosquitoes per a certain area of the territory, but also counting the number of ovipositions.

Beklemishev [1970] realised the difficulty of determining the absolute number of mosquitos and expressed the opinion about the procedure as a very time-consuming and sometimes impossible task mainly because oviposition counting, as a necessary step in determining the absolute number, is a poorly developed method although its practical importance is very high. The accurate counting of mosquito eggs through a potential water-body can predict the larval population size in the future, which can be of great significance, for example, in organising larval control measures. If we know the population size of adult mosquitoes and the previously determined number of ovipositions, we can determine the percentage of viable eggs [Service, 1976; Nikolaeva, 1980].

Eggs can be detected by detailed examination (using a magnifying glass) of a sample material taken from breeding sites (*e.g.*, topsoil or a layer of old decomposed leaves). However, the most efficient and reliable method is to take samples from expected breeding sites and then to analyse them for possible eggs or larvae. Samples can be stored in coolers at +4 °C and examined as needed. Examination can be done manually by rinsing samples with a strong jet of water through a system of sieves [Markovich, 1974; Service, 1976, 1993].

The scientists have found ovipositions of the northern mosquito species *Aedes nigripes* (Zetterstedt, 1838), *Ae. impiger* (Walker, 1848) in moss thickets at river banks, *Ae. communis* (De Geer, 1776) among old decomposed leaves on the bottom of a dry water-body [Service, 1993]. However, there is no full confidence in the reliability of quantitative data obtained by this method, as the survey may not be carried out qualitatively enough. In our opinion, the determination of species composition by collected eggs grows in reliability if larvae of older age are raised from eggs in the laboratory conditions.

Little attention is paid to the study of mosquito oviposition sites, probably for a number of reasons, primarily because eggs of most mosquito species, with the exception of the *Aporheles* genus, are morphologically indistinguishable. Therefore, it is virtually impossible

to estimate truly the proportion of eggs of different mosquito species in a given sample. It is also very difficult to determine the community of mosquito species because the terms of mosquito development phases are known to be different; only in exceptional cases and under certain weather conditions they can coincide. For example, when the spring lasts long the development of early-spring mosquito species occurs simultaneously with mid-spring and typical summer Culicidae species. In such exceptional cases, a very high peak of food activity of female mosquitoes occurs during the warm period of the summer season (June, July) after the long-lasting cool spring period (April, May).

There are a number of methods for detecting and sampling ovipositions by hand. However, almost all of them are very labour intensive so not popular among scientists. According to the assessment of many foreign researchers, one of the efficient semi-automated methods of egg detection is the Horsfall method [Service, 1993]. Taken samples are passed for egg detection through a device designed in a certain way (Fig. 1).

Three cylindrical metal sieves with mesh sizes of 4, 8, 18, respectively, form a shaft through the centre of which (the inner opening of the sieves) a rod with a handle is passed to rotate cylinder sieves. Below the shaft, there is a vessel (tub) with water, through which the lower half of the sieves passes during rotation. The operator rotates the sieves at a speed of 50 revolutions per minute, first in one direction, then in the opposite direction. Eggs together with small soil particles are washed away into the tub, and then the contents of the tub are passed through a hole in its bottom through a system of three sieves put one above the other with mesh sizes of 40, 60 and 120 (mesh size indicates the number of mesh holes per 1 linear inch (25.4 mm), the larger the mesh size, the smaller the size of the particles passing through them). From the third lowest sieve, the eggs are transferred to a vessel below. Their further separation from soil particles and detritus proceeds by flotation using the corresponding chemical compounds, such as NaCl, $MgSO_4$ solutions, etc. Though highly efficient, this method is believed to be difficult and expensive. The author recommends the Salt & Hollic method she personally applies [Service, 1993]. This method means using the commercially available standard nematode egg extraction equipment to extract mosquito eggs from a sample. After the samples have passed through the device, the contents are also rinsed through a sieve system. The detection of eggs among soil particles is carried out similarly to the flotation method described above.

The procedure of revealing the population of blood-sucking mosquitoes by their ovipositions is a hard work. More efficient and reliable is the method of taking soil or vegetation samples from the potential breeding sites, followed by flooding them with water in order to identify larvae hatched from eggs, provided that the eggs are not in diapause. This method has also been used by many foreign researchers [Service, 1993].

Sampling is performed using a metal frame with a side of 20 cm and an area of 400 cm^2 [Becker, 1969]. The further procedure is similar to that described above.

This method of sampling for ovipositions has been also described by the Ukrainian researchers [Shumkov, 1969, 1987]. Samples, a 20 x 20 cm square of soil (from a 3–5 cm depth), were taken using a sharp metal frame or cut out with a knife in various plant communities. Minimum 5 samples were taken from each plant community. The research was carried out in autumn during the period of complete drying of water reservoirs and the absence of mosquitoes in nature. In February and March, samples from storage sites (freezers) were brought to the laboratory, placed in trays and covered with water. The larvae hatched were counted daily, raised to the III–IV age classes, and determined to species. Dubitsky used this determination method of ovipositions [1979].

Quantitative evaluation of mosquito larvae and pupae

The methods for quantitative collection of larvae are better developed than the methods for quantitative collection of mosquito eggs, but the methods are difficult to unify and calibrate. The terms of larval sampling during monitoring is also not standardised; Gutsevich et al. [1970] recommended to survey typical water-bodies every 10–14 days and every week in the South (Caucasus and Central Asia) throughout the whole warm season. We also tested decadal collections of larvae for multi-

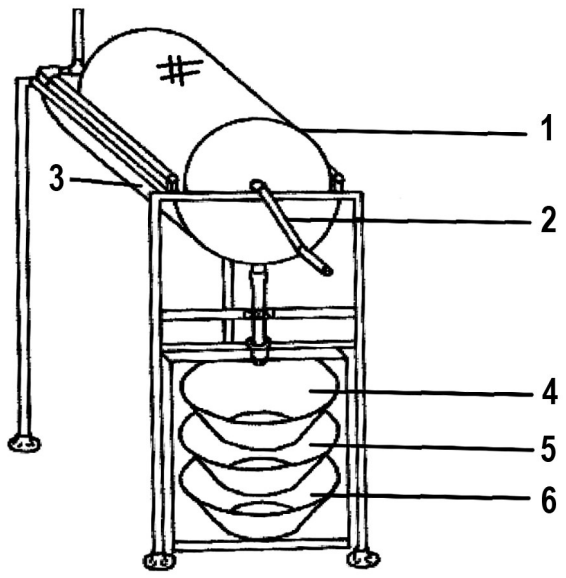

Fig. 1. Horsfall's soil washing machine for collecting mosquito eggs [by: Service, 1993]. Designations: 1 — metal drums with sieves of different mesh sizes (4, 8 and 18), 2 — handle on a rod with a handle for rotating drums, 3 — bath with water for washing the contents of the drums, 4 — sieve with mesh size 40, 5 — sieve with a mesh size 60, 6 — sieve with mesh size 120.

Рис. 1. Прибор Horsfall для обнаружения яиц комаров [по: Service, 1993]. Обозначения: 1 — металлические барабаны с ситами разной величины ячей (4, 8 и 18), 2 — ручка на стержне с рукояткой для вращения барабанов, 3 — ванна с водой для промывки содержимого барабанов, 4 — сито с величиной ячей 40, 5 — сито с величиной ячей 60, 6 — сито с величиной ячей 120.

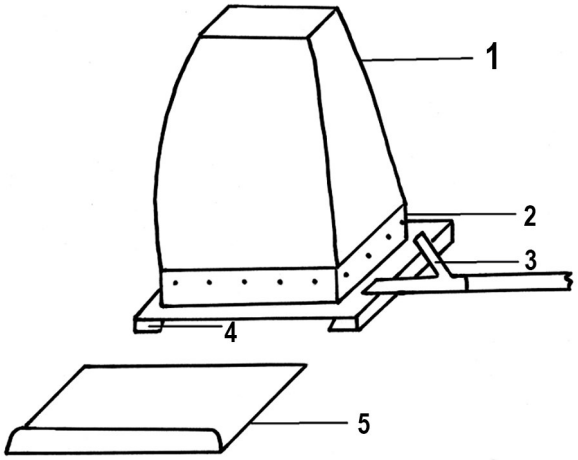

Fig. 2. Water trap for quantitative collection of mosquito larvae and pupae Sazonova's net-frame [Sazonova, 1960]. Designations: 1 — net from mill gas, 2 — a metal frame 25x25 cm with holes (points in the figure) for sewing on a net, 3 — net handle up to 1.5 m, 4 — grooves for a metal valve with a cutting edge, 5 — a metal valve with a cutting edge (used to cut aquatic plants when closing the trap).

Рис. 2. Водная ловушка для количественного сбора личинок и куколок комаров «Сачок-рамка Сазоновой» [Sazonova, 1960]. Обозначения: 1 — сачок из мельничного газа, 2 — металлическая рамка 25х25 см с отверстиями (точки на рисунке) для пришивания сачка, 3 — ручка сачка до 1,5 м, 4 — пазы для металлической задвижки с режущим краем, 5 — металлическая задвижка с режущим краем (служит для подрезания водных растений при закрытии ловушки).

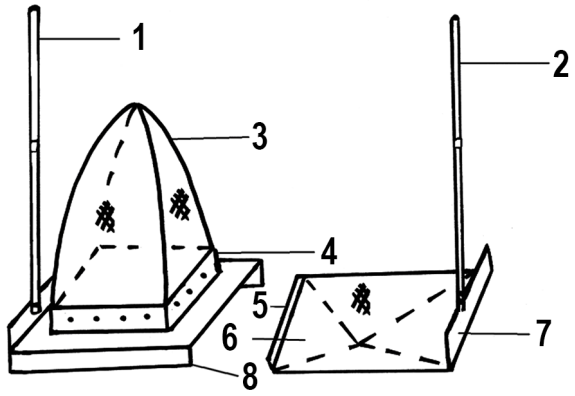

Fig 3. Net-frame for the quantitative collection of larvae and pupae of mosquitoes (original). Designations: 1 — telescopic handle up to 3 m, attached to the frame with the dome of the net (first part of the trap, sinks to the bottom of the first), 2 — telescopic handle up to 3 m, attached to the gate valve (second part of the trap with a flat net, lowered after the first part), 3 — the dome of a net 50 cm long from a veil, 4 — the base of the net, sewn to the frame (point-holes for attaching the net), 5 — cutting edge of the gate valve; 6 — flat net sewn to a metal frame, 7 — metal frame 24x26 cm, 8 — gate slots, metal frame 25x25 cm.

Рис. 3. Сачок-рамка для количественного сбора личинок и куколок кровососущих комаров (оригинальная разработка на основе «сачка-рамки Сазоновой» [Sazonova, 1960]. Обозначения: 1 — телескопическая ручка до 3 м, прикрепленная к раме с куполом сачка (первая часть ловушки, опускается на дно первой), 2 — телескопическая ручка до 3 м, прикреплённая к задвижке-сачку (вторая часть ловушки с плоским сачком, опускается следом за первой частью), 3 — купол сачка длиной 50 см из вуали, 4 — основание сачка, пришитое к раме (точки на рисунке — отверстия для пришивания сачка), 5 — режущий край сачка-задвижки, 6 — плоский сачок, пришитый к металлической раме, 7 — металлическая рама 24х26 см, 8 — пазы для задвижки на металлической раме 25х25 см.

year monitoring of the highly-abundant mosquito *Aedes communis* De Geer, 1776 [Panyukova, 2022].

Native researchers prefer the method of collecting and counting mosquito larvae with a hoop net. When describing the method of counting larvae and pupae, the authors refer to the Monchadsky method [Monchadsky, 1952]. According to this method, mosquito larvae are collected by a slanted (at an acute angle) submerged hoop net (diameter 20 cm) made of gauze or silk bolting cloth from a distance of 1 m of water surface first in one and then in the opposite direction. To obtain the data on the abundance of larvae per 1 m² of water surface, the received number of larvae should by multiplied by 5, since covering a distance of 1 m of water surface with a 20-cm-in-diameter net corresponds to 1/5 m² [Vinogradskaya, 1972]. By the later interpretation of the Monchadsky method [Gutsevich et al., 1970], the use of gauze for netting is completely excluded because of the high probability of insufficient catch of juvenile larvae. At now, there is a wide range of modern fabrics with the required properties to ensure the efficiency and completeness of collections: dense capron, dense organza [Panyukova, Bespyatova, 2013], tulle, veil, sieve cloth, polyester nets with fine meshes.

Following the decision of the scientific supervisors N.A. Violovich and A.I. Cherepanov how to analyse blood-sucking insects in Siberia in 1960-ies, the scientists of the Institute of Animal Systematics and Ecology of the Siberian Branch of the Russian Academy of Sciences (formerly the Biological Institute of the Siberian Branch of the USSR Academy of Sciences) adopted the lower described method. Larvae were collected using an entomological hoop net with a diameter of 20 cm and a depth of 25 cm. Then, the obtained number of larvae in a sample (or per area of net) was recalculated by multiplying by 16 to receive the number of larvae per 1 m². By the results of long-term studies of mosquito species composition and ecology in the forest and forest-steppe zones of Siberia, in particular, in the vicinity of Novosibirsk and adjacent areas of the Novosibirskaya Oblast, using two larval collection methods as with a net being 20 cm in diameter and standard cuvettes of different sizes we have identified dominant species in the mosquito community. By the number of preimaginal phases of these species (taking into account certain weather conditions), it is possible to predict their population size in the study region.

To count larvae in water-bodies with a flat bottom, the Sazonova net-frame is used [Sazonova, 1959; Rasnitsyn, 1974]. The frame has a net in form of a bag, as well as grooves through which the lid, which closes the neck of the net, moves. The frame is placed in the bottom of the water-body, the lid is closed manually, frame dimensions are 25x25 cm, handle length is 1.5 m (Fig. 2). The number of caught larvae and pupae is recounted for 1 m²; this trap catches larvae in a water column with a cross-section of 1/16 m² [Sazonova, 1959].

On the basis of the Sazonova net-frame we have developed a modified trap for collecting samples in deep water-bodies. It consists of two parts; each part has a long handle (Fig. 3).

The handles of the trap are located not horizontally, like those of the Sazonova net-frame, but vertically. The first part of the trap is a metal frame with a long hoop net at the top and with skids-grooves it the bottom, into which the second part of the trap is inserted. The second part of the trap is a frame-net-slider with a cutting edge. The hoop net of the second part of the trap is flat and small; it has a small sag of cloth in its centre. Both parts of the trap have handles made of strong lightweight material, which extend up to 3 metres. The dimensions of the first part of the frame are 25x25 cm and the length of the hoop net is 50 cm. The second part of the trap is a slider made of a metal frame with a cutting edge, with dimensions 23x26 cm, lined with a hoop net.

The operation with the trap starts with lowering the frame with a long net onto the bottom of the water-body with a sharp movement, so that a water column of 1/16 m² is covered. Then the second part of the trap is lowered to the bottom of water reservoir and inserted by the handle into the grooves located in the first part of the trap. The net-frame trap is then raised up to the surface by two handles. The larvae and pupae remain on the net of the sliding frame as the water is filtered through the large hoop net of the first frame. A test sample of the device was made of metal wire (frame), plastic tubes (handles of the trap parts) and white veil (nets). The device was tested in the flood meadows of the Vychegda River (Komi Republic) as an anophelogenic water-body typical of the region (Fig. 4). For qualitative collection of mosquito larvae, especially malaria larvae, from the surface of the water-body, the second part of the trap with a flat hoop net is recommended for use.

The other authors Nikolaeva, Olshvang [1978] proposed the method of collecting and counting mosquito larvae using a standard biocenometer in form of a square tube made of roofing iron or other metal, each side of which is 25 cm wide. The sharp lower edges of the biocenometer are pressed into the ground, thus cutting off a water column with a base area of 1/16 m². The caught larvae get out of the tube within a small net with a 12x12 cm rectangular hoop. The number of caught larvae is then recounted for 1 m². The authors believe that if the larval density is low (50 specimens per sample and less) it takes 25 minutes to collect larvae, if the larval density is high (up to 400 specimens per sample) — 10–12 minutes [Nikolaeva, Olshvang, 1978]. In small water reservoirs (10, 15 m² and less), one counting procedure includes 1–2 samples, in larger water-bodies (200–400 m²) — 5–7 samples. Knowing the density of larvae per 1 m² of a water-body and multiplying it by the whole area of the water-body, we can get the absolute number of larval population on a certain territory [Nikolaeva 1978, 1980].

According to Vansulin [1977], the absolute population size of mosquitoes can be determined by the number of pupae. The reliability of the chosen method is confirmed, in the author's opinion, by the fact that the dynamics of pupae per unit of counting and mosquitoes per unit of time (20 minutes of counting on his/her own body) coincide. The other authors [Mamedniyazov et al., 1988] counted larvae using an entomological hoop net with a diameter of 20 cm and a depth of 25 cm. Then the larvae collected in a sample (per area of a net) were multiplied by 31.7. This value, as the authors believe, allows for recalculating the obtained number of larvae for 1 m² (the coefficient is the number of net areas equal to 1 m²).

Fig 4. Testing of the modified net-frame.
Рис. 4. Апробация модифицированного сачка-рамки.

In the book 'Mosquito ecology: field sampling methods' [Service, 1993], the author describes a number of methods for collecting and counting mosquito larvae, including semi-automatic devices. To our mind, only few of them are more or less suitable for forest and forest-steppe zones and are worth attention, especially in view of the climate warming trend and reduction of areas appropriate for mosquito breeding. Two semi-automatic collection methods can be used in shallow water-bodies with further recalculation of larvae per unit area.

The area sampler for counting larvae per unit area, Roberts & Scanlon [Service, 1993]. The sampler consists of two plastic cylinders up to 30 cm high (Fig. 5). The outer cylinder has a diameter of 12.5 cm and the inner cylinder has a diameter of 11 cm. The bottom of the inner cylinder is a plastic hollow cone cut at an angle of 67 degrees (inverted plastic funnel). The small diameter of the funnel has a hole into which a lightweight rubber plug is pulled up during operation to close it. The plug is tied to a cord. The cord is attached to a crosspiece placed across the top hole of the inner cylinder. The outer cylinder is carefully drilled into the ground of the water reservoir, then the inner cylinder is also carefully inserted into it.

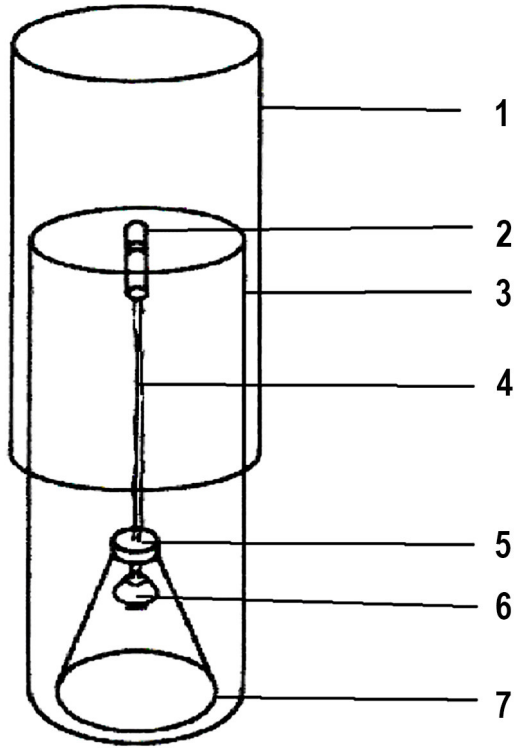

Fig 5. An area sampler for assessing the number of mosquito larvae [by: Roberts, Scanlon, 1974]. Designations: 1 — external cylinder 30 cm high, 12.5 cm in diameter, 2 — a jumper in the inner cylinder, to which a cord with a cork is attached, 3 — inner cylinder with a diameter of 11 cm, 4 — cord with rubber stopper, 5 — hole in the inner cylinder, 6 — rubber plug on the cord, 7 — cone-shaped hole in the inner cylinder.

Рис. 5. Прибор (пробоотборник) для оценки численности личинок комаров [по: Roberts, Scanlon, 1974]. Обозначения: 1 — внешний цилиндр высотой 30 см, диаметром 12,5 см, 2 — перемычка во внутреннем цилиндре, к которому крепится шнур с пробкой, 3 — внутренний цилиндр диаметром 11 см, 4 — шнур с резиновой пробкой, 5 — отверстие во внутреннем цилиндре, 6 — резиновая пробка на шнуре, 7 — конусовидное отверстие во внутреннем цилиндре.

After some exposure (15–20 min.), when larvae from the outer cylinder together with water reach the inner cylinder through the funnel-shaped bottom, the cork is pulled up closing the funnel opening. The contents of the inner cylinder are then poured into a container, from which the larvae are extracted, counted, recorded, etc. The authors of the method believe that the device is very useful and reliable, but has certain disadvantages and limitations. When used in forest water-bodies, the funnel opening of the inner cylinder becomes quickly clogged with debris (leaves, needles, detritus, etc.). The authors recommend that, before placing the inner cylinder into the outer cylinder, large debris particles should be previously removed from the water surface. When used in shallow water-bodies with a water depth less than 2.5 cm, the inner cylinder is recommended to be refilled with water (obviously excluding larvae introduction). Taking into account the problems associated with the described method as a long waiting period for larvae rising within a water flow through the funnel into the inner cylinder (15–20 minutes), limited use only in shallow water- bodies, the authors of the method recommend to use up to 10 devices simultaneously to solve these problems and are sure that they are quite simple repeat.

To overcome these problems, Taylor [1979] proposed a modified version of the sampler developed by the above-mentioned authors (Fig. 6). It also consists of two plastic cylinders, the outer one with a diameter of 12.05 cm and a height of 33 cm and the inner one with a diameter of 10.5 cm and the same height. The lower edge of the outer cylinder is enclosed in a ring of a 3-cm-wide steel strip with a sharply serrated lower edge to facilitate its insertion into the ground of the water reservoir (it can cut through a layer of leaves, thin tree branches, etc.). A bottom end is inserted into the inner cylinder. It consists of two plastic round plates, between which a rubber circle is inserted in order to hold the bottom end firmly in the lower edge of the cylinder. There is a hole with a diameter of 3.6 cm in the centre of the bottom end. On the inside, the edge of this hole is enlarged (bevelled) for a better fitting of the rubber plug, which is used to plug the hole during operation. In practice, the outer cylinder is firmly planted in the pond, then the inner cylinder is carefully inserted into it. Through the hole in its bottom water flows up into the inner cylinder. After all water with larvae has passed the hole and reached the inner cylinder, the hole in the bottom is plugged with a rubber plug attached to a strong metal rod. Two crosspieces are attached to the rod to prevent it from deflecting and to help positioning the plug correctly into the bottom hole in turbid water conditions. The operation with this device required considerably less time. The entire procedure, according to the author, takes about two minutes [Taylor, 1979].

The construction of this device is not difficult. It can be easily prepared, for example, of two cut plastic bottles, each being 33 cm long made of durable plastics, or plastic tubes with a diameter of 12 and 10.5 cm. After installing the cylinder of a larger volume onto the bottom of a shallow pond, the second bottle, inverted with the neck downwards, should be pushed slowly (within a minute) almost to the bottom (the neck forms a funnel through which the water with larvae will enter the bottle of a smaller volume) according to the principle of a piston. Then, remove the outer cylinder and close the neck from below with a rubber stopper or a cap from a suitable bottle. The use of this construction will greatly facilitate and speed up the process of catching mosquito larvae, simultaneously ensuring the reliability of the obtained quantitative data. Recalculation is also done per 1 m^3 or per 1 litre of water.

In hydrobiological practice, the scientists apply different batometers for quantitative sampling, with subsequent filtration of water through a plankton net. Batometers are good because they allow obtaining samples from the depth required by the researcher. However, using a bathometer does not exclude the possibility of undercounting fast-moving organisms trying to escape from the device. In littoral and shallow water-bodies, samples are collected by pouring 50–100 litres of water with a 10-litre bucket through a plankton net [Kononova, Fefilova, 2018].

To illustrate the calculations, let us give an example. A 50-litre water sample is taken (5 buckets of water are poured through a filter (a plankton net or a hoop net)), then we count the number of mosquito larvae in the sample. For example, 5 mosquito larvae are found in the sample (50 litres). Then, the obtained value is recalculated for 1 m^3 (1 m^3 — 1000 litres), the coefficient for such a sample is K = 20 (1000 litres : 50 litres). The number of specimens in the sample is multiplied by the obtained coefficient, the number of larvae in the given water body is 100 specimens / m^3 (5 specimens x 20).

Discussion

In our opinion, all the above-listed methods of larval counting suffer from limitations, as they are not applicable to different water-bodies. For example, temporary water-bodies do not always allow to carry out work with a hoop net covering 1 m of water surface, because the size of temporary water-bodies often does not exceed 1 m in diameter. This method is suitable for large temporary or permanent, especially anophelogenic, water-bodies.

The Sazonova frame can be used only in shallow flat-bottomed water-bodies, as the trap is manually closed, which significantly limits its use at large depths. The Sazonova modified frame is possibly designed for use in deeper water-bodies with steep banks but not deeper than 3 metres [Sazonova, 1959]. Its disadvantage is the limited depth and poor visibility when combining the trap's parts in the water column.

The biocenometer can give a true picture on the larval density in certain areas, such as sedge tundra, moss tundra or forest tundra, as well as in vast peatlands and marshes of Eastern Siberia but it is practically impossible to use it when counting larvae in the taiga and forest-steppe zones. It is often difficult to place the biocenometer into the ground because of branches, twigs, roots of trees and bushes lying on the bottom. Gaps occur and larvae are undercounted. The same difficulties in working with this counting device were mentioned earlier [Service, 1993]. Various natural or artificial water-bodies, sometimes very deep pits, ditches, and quarries, which, according to our observations, in some years are filled with larvae of mosquitoes of the *communis* and *cantans* groups, are often inaccessible for larval counting with a biocenometer [Mirzaeva, Glushchenko, 2008; Mirzaeva, Khodyrev, 2013]. Larvae in such water reservoirs can be counted only ashore using a long-handled hoop net or the modified Sazonova net-frame. In addition, any bulky instruments that are difficult to transport or expensive devices can be placed only in areas protected from vandalism, i.e. at the corresponding scientific stations, in nature-protection reserves.

Standard-sized cuvettes (18x22; 13x19) and a water hoop net with a diameter of 20 cm appeared to be very convenient and quite reliable collection methods for determining the density of mosquito larvae in water-bodies. These collection and quantification tools are applicable in any landscape zone during route surveys of water-bodies as they are easy to use and transport (both the net and the cuvette). Their use in different landscape zones allows for comparative analyses of mosquito abundance at the preimaginal phases. In our opinion based on many years of practice, collection with a 25-cm-diameter water hoop net remains a highly promising determination method of mosquito larvae, as Rasnitsyn also expressed in his work [Rasnitsyn, 1974], because it meets the criterion of stability. The net quickly covers a water area of 25 cm^2 (a quarter of 1 m^2). Moreover, the net does not scare away larvae during its rapid immersion into water as it happens during the forward and backward movement of net according to the Monchadsky method. When we

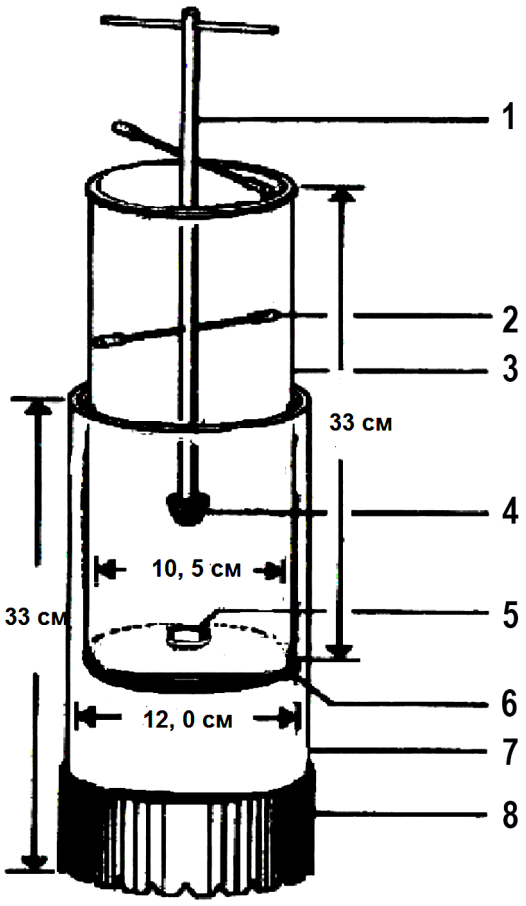

Fig. 6. An area sampler for assessing the number of mosquito larvae [by: Taylor, 1979]. Designations: 1— Metal rod, serves to close the device, 2 — jumpers, serve to center the rod when closing the cork, 3 — inner cylinder made of plastic, 10.5 cm in diameter, 4 — rubber stopper at the end of the rod, 5 — hole in the bottom of the inner cylinder, 6 — the bottom of the inner cylinder of two plastic plates, between which there is a rubber circle, 7 — external plastic cylinder with a diameter of 12 cm, 8 — base.

Рис. 6. Прибор (пробоотборник) для оценки численности личинок комаров [по: Taylor, 1979]. Обозначения: 1 — Металлический стержень, служит для закрывания прибора, 2 — перемычки, служат для центрирования стержня при закрытии пробки, 3 — внутренний цилиндр из пластика, диаметром 10,5 см, 4 — резиновая пробка на конце стержня, 5 — отверстие в донышке внутреннего цилиндра; 6 — донышко внутреннего цилиндра из двух пластиковых пластин, между которыми имеется резиновый круг, 7 — внешний пластиковый цилиндр диаметром 12 см, 8 — основание.

cover one metre of water surface by the hoop net according to the Monchadsky method, the obtained estimate of larval density is a very approximate number compared to that when we cover a quarter of water surface because it is often difficult to visualise how to operate the net to cover accurately a length of 1 m.

Long-term studies with the same counting methods allow for monitoring changes in the mosquito community structure and drawing valid conclusions about the impact of various factors, including the climate change. *E.g.*, having used the same methods, we have found a reliable increase in number of polycyclic mosquito species [Mirzaeva, 2008; Mirzaeva, Khodyrev, 2013] in the vicinity of Novosibirsk and adjacent areas of the Novosibirskaya Oblast since the late 1990s till today.

In general, all the above methods shed light on the relative abundance of larvae. The quality of any counting method is also affected by various subjective and objective negative factors, such as weather conditions, the collector's dexterity, and the effect of scaring away larvae when the net, biocenometer, or sampler is immersed into water. At present, there is no method for determining the absolute mosquito abundance but there are methods that can accurately estimate the mosquito abundance at the preimaginal development phase. Using these estimates, we can forecast the adult mosquito abundance in the current season. To monitor the seasonal and inter-seasonal dynamics of mosquito population in the particular study area, it is important to choose adequate corresponding methods and use them continuously to exclude the influence of the method on the results. The choice for this or that quantitative method of mosquito collection at the preimaginal stage depends, in our opinion, on the type of water-body. The table below highlights the classification type of water-bodies, the recommended method of larval collection, and the frequency of quantitative counts of larvae and pupae of Culicidae (Table 1). This classification of water-bodies relies on the characteristics being important for the right choice of the quantitative collection method as duration of existence (permanent and temporary), type of water movement (stagnant and flowing), bottom type (flat or uneven), and depth (deep (more than 1 m) and shallow (less than 1 m)). We do not take into account only the material the bottom is composed of (sand, stone, silt) and the degree of plant growing in water-bodies. These qualities of a water reservoir are important for the inhabitants of the aquatic ecosystem but useless for the choice for a collection method, as they make any collection tool difficult to operate.

The work on improving the methods for collecting and quantifying mosquito larvae and pupae, solving the task how to get information on the absolute mosquito number, will be continued.

Acknowledgements

We sincerely thank T.A. Novgorodova for valuable comments and corrections to the manuscript. We would like to thank N.E. Panyukov for his help in developing the layout and preparing the trial sample of our device for collecting mosquito larvae. The work of A.G. Mirzaeva was financially supported by the project of the Fundamental Scientific Research Program, state № FWGS-2021-0002. The work of E.V. Panyukova was carried out within the frames of the research project of the Department of Animal Ecology at the Institute of Biology Komi SC UB RAS № 125013101229-9.

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Table 1. Correspondence of the type of water-body to the recommended method of quantitative collection of preimaginal stages (larvae and pupae) of mosquitoes during the seasonal monitoring

Таблица 1. Методы количественного учёта преимагинальных фаз (личинок и куколок) кровососущих комаров, рекомендуемые для мониторинговых исследований в водоёмах разного типа

Type of water-body	Recommended method	Number of counts per month
Permanent stagnant shallow-water (no more than 1 m deep) with flat bottom (pit, quarry, lake, flood meadow, marshland)	Sazonova net-frame, water hoop net, Nikolaeva biocenometer	3–7
Permanent stagnant deep-water (more than 1 m deep) with flat bottom (pit, quarry, lake, flood meadow, marsh)	Modified Sazonova net-frame, water hoop net	3
Permanent flowing deep-water (more than 1 m) with uneven bottom (ditch, river, lake, stream)	Modified Sazonova net-frame, water hoop net	3
Temporary flowing deep-water (stream, ditch)	Modified Sazonova net-frame, water hoop net	3
Permanent flowing deep-water (river, stream)	Water hoop net	3
Temporary stagnant shallow-water (puddle, hole, swamp, water tank, pond in tree trunks, flooded house cellars, ponds between hummocks in peatlands)	Dippers, cuvettes, samplers	3–5
Temporary stagnant deep-water (pit, marsh)	Modified Sazonova net-frame, water hoop net	3–7
Permanent stagnant shallow-water (fire pond)	Modified Sazonova net-frame, water hoop net	3–7

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Поступила в редакцию 5.5.2023