Report

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Distribution and Dynamics of Larval Populations of *Anopheles messeae* and *A. atroparvus* in the Delta of the Rivers Rhine and Meuse, The Netherlands

The distribution and ecology of mosquitoes of the *Anopheles maculipennis* complex were studied in the delta of the rivers Rhine and Meuse in the southwest of The Netherlands. The study area was previously malarious, with *A. atroparvus* being the only vector. 125 potential aquatic habitats of *A. maculipennis* were sampled, of which 47 (37.6%) contained larvae of this species complex. Larval densities varied from 7.4–325.93 larvae m^{-2}. There was no correlation between chlorinity (‰) of the water and presence and/or density of larvae. The presence of *A. maculipennis* was not associated with one particular aquatic floristic habitat, although larvae were often found together with floating algae (*Enteromorpha* spp.). Larvae were not found in areas experiencing tidal flooding. A newly developed PCR method was used for identification of the mosquito sibling species. Of 150 larvae examined, only 4 were identified as *A. atroparvus*. All other larvae examined were *A. messeae*. Adult mosquitoes were identified as *A. messeae* and 30 wild-caught mosquitoes had fed on domestic animals. Because most anophelines found in 1999 were *A. messeae*, it is concluded that the study area has undergone a dramatic ecological change since the previous anopheline investigations in 1935, causing the near extinction of *A. atroparvus*. This species was the only malaria vector in The Netherlands and therefore it is not expected that malaria can return to its former endemic status in the coastal areas of The Netherlands.

Typical drainage ditch in grassland near Vlaardingen, South Holland. The water was brackish (800 mg Cl L^{-1}) with a vegetation that supports *Anopheles messeae*. Photo: W. Takken.

INTRODUCTION

During the first half of the 20th century malaria was common in the coastal provinces of The Netherlands. For example, in Amsterdam more than 2400 cases of malaria were registered in 1946 (1). The most prevalent parasite was *Plasmodium vivax*, but at least during the first decade of the century *P. malariae* was also present. The disease was transmitted by the mosquito *Anopheles atroparvus* Van Thiel. *A. atroparvus* is a member of the *A. maculipennis* complex, which in The Netherlands further consists of *A. messeae* Falleroni and *A. maculipennis* s.s. Meigen (2, 3). Other anopheline species that have been recorded from The Netherlands are *A. claviger* and *A. plumbeus*. Of these, only *A. plumbeus* has been incriminated as a malaria vector in western Europe. *A. claviger* is a vector in the Mediterranean (4). In The Netherlands the disease was limited mostly to the coastal provinces because of the optimal breeding conditions for *A. atroparvus* in brackish waters (5). Further inland, in less brackish or freshwater systems, *A. atroparvus* could not develop into the high-density populations required for malaria transmission. Apart from the uniqueness of the single vector, there was one more typical character associated with malaria transmission in The Netherlands: people became infected with the parasites by the bite of mosquitoes in the autumn (September–October) but developed the disease only 8–10 months later (May–July), unlike in the tropics where the incubation time of the disease is only 2 weeks (6). The onset of the disease in the early summer coincided with the emergence of the vector mosquitoes from hibernation. As is common with this type of *P. vivax*, many people were asymptomatic carriers and served as an infectious reservoir for the mosquitoes in September–October. Immediately following World War II, anti-malaria activities were undertaken by indoor spraying with insecticides (DDT) and active surveillance and treatment of *Plasmodium* carriers with quinine. The last case of indigenous malaria in The Netherlands was reported in 1961 (1, 7).

During the second half of 20th century, major engineering works in The Netherlands caused the transition of many brack-

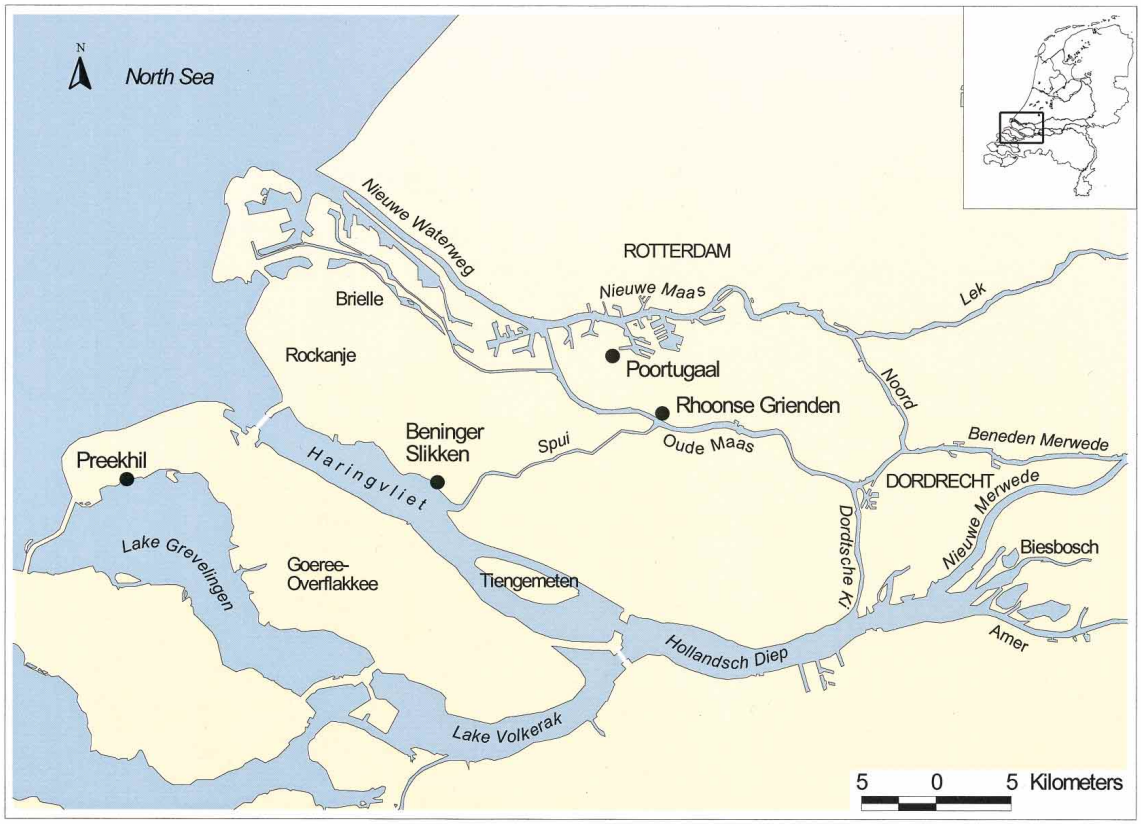


Figure 1. Map of the study area and relevant locations that are mentioned in the text.

ish waters to freshwater systems. Also, the introduction of synthetic detergents caused heavy pollution of mosquito larval habitats. It is believed that both developments combined caused the near extinction of *A. atroparvus* from The Netherlands, and with it the continued risk of malaria transmission (8). However, during the last decades of the 20th century, anti-pollution measures and a strong interest in environmental conservation resulted in a strong improvement of the water quality. In addition, plans have been developed to restore some of the formerly brackish waters to their original state by allowing seawater to re-enter wetland areas (9, 10). Thus, the ecological conditions under which *A. atroparvus* once thrived, are expected to return. The question is, will this be followed by a recovery of *A. atroparvus* populations and, consequently, a return of malaria risk in The Netherlands?

The lower delta of the Rhine and Meuse rivers was malarious up to World War II (5) and malaria vectors were common throughout the delta (2). The present study was undertaken to investigate the current distribution of the malaria mosquito and its population dynamics in the delta of the Rhine and Meuse rivers in the southwestern part of The Netherlands, with particular emphasis on the ecology of the aquatic mosquito population. The results will be discussed considering the likelihood that occasional outbreaks of malaria might occur in The Netherlands as a result of adequate anopheline mosquito densities.

DESCRIPTION OF THE STUDY AREA

The study was conducted in the lower delta of the rivers Rhine and Meuse, south of Rotterdam. To the west, the study area is bordered by the North Sea and to the north and east by the rivers Noord, Beneden Merwede, Nieuwe Maas and Nieuwe Waterweg. The rivers Nieuwe Merwede and Hollandsch Diep, Lake Volkerak and Lake Grevelingen form the southern limit (Fig. 1). The study area comprises 1500 km^2 and is intersected by many small and large streams, lakes and waterways. As much of the land is below sea level, ditches, and canals have been created to drain the area permanently by using pumping stations. The artificial drainage of the area causes an enhanced seepage of seawater into surface waters. The Nieuwe Waterweg, Oude Maas, Hollandsch Diep and Haringvliet are the most important waterways in the area, carrying the bulk of the water from the Rhine and the Meuse to the North Sea. In the study area, there were originally 3 open-sea arms: the Nieuwe Waterweg, the Haringvliet and the Grevelingen/Volkerak. To protect the southwest of The Netherlands from catastrophic coastal flooding, as occurred in 1953, the "Delta Plan" was made to provide for the closure of all tidal inlets in the study area, with the exception of the Nieuwe Waterweg. In 1970, the Haringvliet was closed off from the sea by means of a dam with outlet sluices. As a result, the Haringvliet turned into a freshwater river system with a strongly reduced tidal range (about 30 cm). In 1972, the Grevelingen was closed off from the sea and the Volkerak was embanked from the Grevelingen and Oosterschelde in 1965 and 1987, respectively. This resulted in a brackish/salty Lake Grevelingen and a freshwater Lake Volkerak. The coastal flatlands behind the dikes along the Haringvliet and Hollandsch Diep, which traditionally contained brackish to salty groundwater, gradually turned to freshwater areas. Indeed, the study area now contains several points where freshwater is being collected for household water supply.

The northern and eastern parts of the study area form part of the urban conglomerate of mainport Rotterdam and are densely populated as well as heavily industrialized. Rotterdam harbor is the world's largest commercial port, with ships entering through the Nieuwe Waterweg. By contrast, the center and southern parts of the study area are remarkably rural and dominated by agricultural activities. The seashores are used for port activities (west of Rotterdam), tourism and/or nature reserves.

Three areas were selected for detailed studies of larval populations. These were Preekhil (5°29'50"E, 42°40'10"N), Brielle (7°02'82"E, 43°44'54"N) and Poortugaal (8°72'00"E, 43°29'50"N) (Fig. 1). The study site in Preekhil is a relatively undisturbed grassland polder adjacent to a nature reserve, far

away from a major town. The site in Brielle was a ditch separating a residential area from dairy grassland. In Poortugaal, the site was located inside a small nature reserve next to an industrial and harbor area. In all sites, dairy cattle grazed within 500 m from the sampling site. In addition, neighboring meadows contained shelters for cattle, horses, and sheep.

MATERIAL AND METHODS

Measurement of Chlorinity

The electrolytic conductivity of larval habitats was measured with a conductivity reader (model WTW - LF196, Retsch, Ochten, The Netherlands). By calibration with solutions of known Cl contents, electrolytic values were transformed to Cl values. For the purpose of this study, we define water as 'fresh' when the Cl content is less than 300 mg L^{-1}. Brackish water has Cl contents ranging from 300–17 000 mg L^{-1}. Water containing > 17 000 mg Cl L^{-1} is considered salty. The Cl contents of seawater are approximately 19 000 mg L^{-1} (11).

Measurement of Temperature, Rainfall and Relative Humidity

The meteorological conditions during the study period were obtained from the Royal Netherlands Meteorological Institute's (KNMI) field station in Wilhelminadorp, approximately 40 km south of the study area. Cloud cover for the areas close to Rotterdam was derived from the meteorological station at Rotterdam Airport. Water temperatures from all sample sites were measured with the conductivity reader at the time of sampling. At the sample site in Brielle the water and air temperatures were measured continuously during the months of August and September using a data logger and temperature probes. The water temperatures collected in Brielle were used to estimate the water temperatures during other times of 1999, using the model developed by Jacobs et al. (12). This model calculates the water temperature at the surface area, i.e. the habitat of anopheline larvae (13).

Mosquito Sampling

Larval stages of mosquitoes were collected by dipping with a white soup ladle; one dip was calibrated to collect 135 cm^2 of surface water. The ladle was gently immersed until the edge was just under the water surface. After filling, it was quickly lifted out and inspected for the presence of mosquito larvae and/or pupae. Depending on the number of larvae collected, 50 or 100 dips were taken per site on each collection day. All larvae and pupae present were collected and sorted. In the laboratory, field-collected anopheline larvae were reared in small pans until 4th larval instar or pupation and emergence of the adult mosquito (14). Larvae were fed Tetramin® fishfood. Adult mosquitoes were collected with aspirators from animal shelters. After morphological identification, blood meals were collected on filter paper and dried over silica gel. Bloodmeal identification was done according to the precipitin method (15). The head and thorax of the mosquitoes were kept for identification by PCR (see below).

Species Identification

Mosquitoes were sorted to species or genus (*A. maculipennis* s.l.) based on larval or adult morphological characteristics. Most larvae were reared to 4th instar and stored singly in 70% alcohol until molecular identification using a PCR technique (16). Some field collected adults of *A. maculipennis* s.l. were also identified by PCR. Ribosomal DNA was isolated from alcohol-stored larvae or dried adults by incubating an entire mosquito in 100 µl Bender buffer (0.1M NaCl, 0.1M Tris-HCl pH 7.5, 0.05M EDTA pH 7.5–8, 0.5% SDS) at 65°C for 30 min, after which 50 µl of pre-chilled KAc was added, mixed well and placed on ice for a further 30 min. Following centrifugation for 5 min at 14 000 rpm, 200 µl of ethanol were added and DNA isolated by precipitation at –20°C for 30–60 min. The supernatant was then removed by pipette and the DNA dried under vacuum for 30 min at room temperature, before re-suspending in 50 µl water and storing at 4°C. Each DNA isolate was diluted by x10 for PCR. The PCR identification employed a Perkin Elmer 480 thermal cycler (Cetus Corp., USA) using the oligonucleotide primers and protocols described by Proft et al. (16). DNA isolates from *A. atroparvus* (Leiden strain, kept in the Wageningen Entomology laboratory), *A. messeae* and *A. maculipennis* s.s. (courtesy of J. Proft, University of Bonn, Germany) were used as controls in each PCR procedure and electrophoresis.

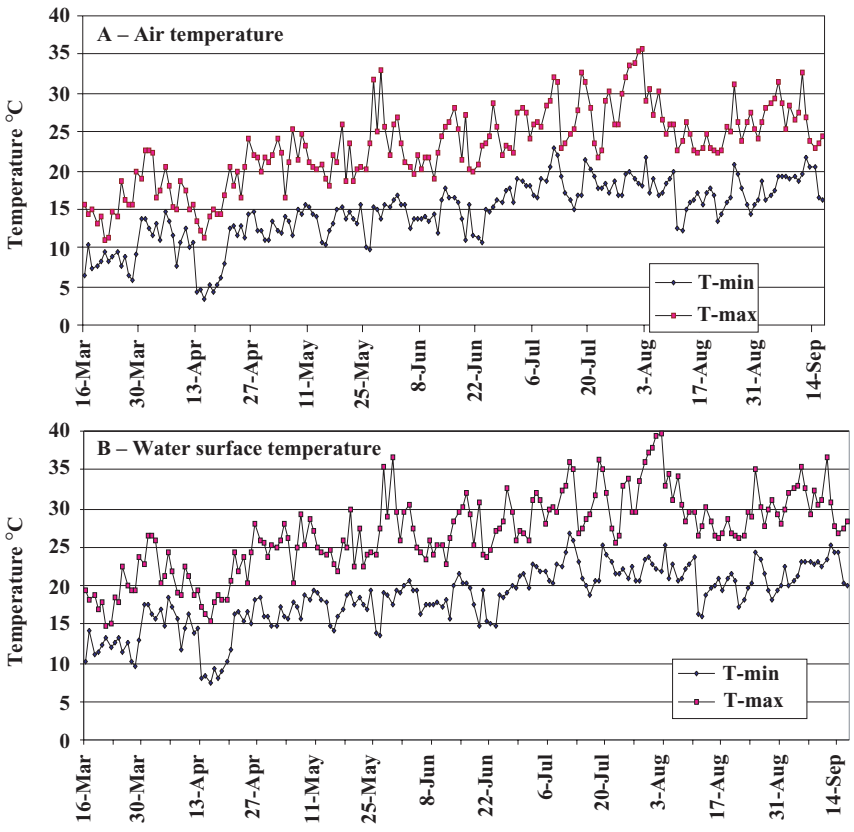
Estimates of Population Density and Development

At the sentinel study sites in Preekhil, Brielle, and Poortugaal, larval collections were made at weekly intervals during August and September to estimate the population density and development. All anopheline larvae were sorted to species and larval stage. Using geographical information about the total water surface area at each study site, the minimum and maximum number of larvae per m^2 and per km^2 of surface water were estimated.

RESULTS

The general survey for the presence of anopheline larvae in the study area was conducted in July 1999. Population studies of *A. maculipennis* in Preekhil, Brielle and Poortugaal were done in August and September 1999.

Figure 2. Minimum and maximum temperatures in Brielle from March–September 1999: (Calculated temp.) A – ambient air, B – water surface.



Meteorological Conditions during the Study Period

In Figure 2 the minimum and maximum temperatures of the ambient air and of the water surface in Brielle are shown from 16 March to 14 September 1999. This period corresponds with the expected activity of adult *A. maculipennis* s.l. in The Netherlands (2). On average, at the surface, the temperature of the water was 2.5°C higher than that of the ambient air. Rainfall during the study period (March–September) was 451.6 mm, with peaks in March (95.4 mm), June (71.3 mm) and August (106.5 mm). In 1999, the months of July and September were relatively dry compared to historical averages. Mean temperatures in July and August were above the historical values, with mean temperatures of 19.1 and 18.3°C, respectively.

Current State of Freshwater and Brackish Waters

Analysis of 125 sites throughout the study area showed that the chlorinity of the waters varied from 100 to 14 838 mg Cl L^{-1}. Sites in direct and open connection with the North Sea and exposed to large tidal changes were salty. The water level at these sites varied with 100–175 cm between tides, depending on the distance from the open sea. This applies to sites along the Nieuwe Waterweg, Nieuwe Maas, and Oude Maas. Formerly salty and/or brackish areas, now closed off from the sea and exposed to continuous flushing with Rhine or Meuse waters, were fresh or slightly brackish. In several polders, ditches contained brackish water while the surrounding surface waters are fresh. This is due to seepage from salt deposits below the surface.

Distribution of Anopheline Larval Habitats and Estimated Densities of *Anopheles. maculipennis* s.l.

Fourty-seven of the 125 sites sampled for anopheline larvae contained *A. maculipennis* s.l. (Fig. 3). In 4 of the 47 sites *A. claviger* was also present, jointly with *A. maculipennis* s.l. Larval densities varied from 0.01–4.40 per dip, equivalent to 7.4–325.93 lar-

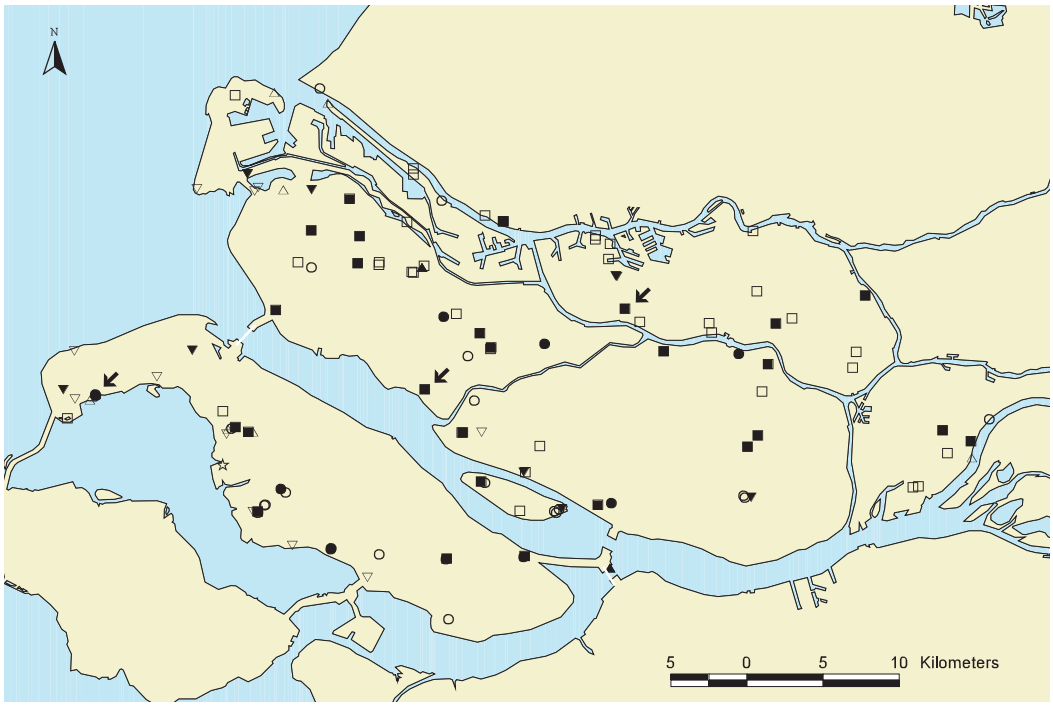


Figure 3. Presence of anopheline larvae in the study area and chlorinity of sampling sites. Arrows indicate the locations where *Anopheles atroparvus* was found. Legends: Open symbols; no anophelines present; Closed symbols; *Anopheles maculipennis* spp. present.
△ 0–300 mg Cl L^{-1}
□ 300–750 mg Cl L^{-1}
○ 750–1500 mg Cl L^{-1}
☆ 1500–17 000 mg Cl L^{-1}
▽ >17 000 mg Cl L^{-1}

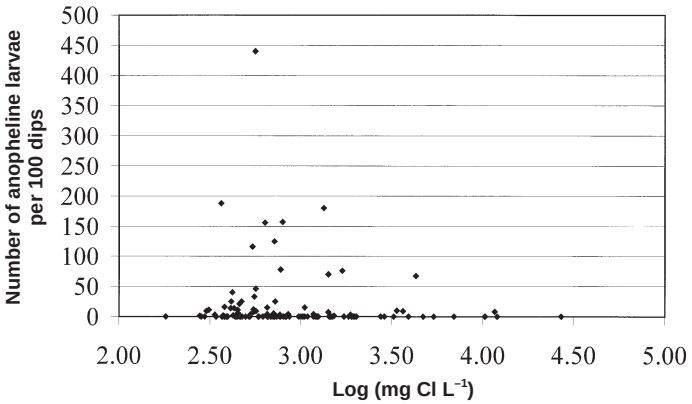
Table 1. Density estimates of larvae and daily emerging adults in Preekhil, Brielle and Poortugaal under scenarios where 30% or 100% of the water surface are suitable for larval breeding.

Location	Water surface (m^2) km^{-2}	No. larvae m^{-2} observed			Estimated no. of larvae km^{-2}		Daily emerging adult mosquitoes km^{-2} (see legend for calculation)	
		min.	max.		minimum	maximum	minimum	maximum
Preekhil	72 567	7.4	118.5	larval area = 0.3 water surface	161 099	2 579 757	38 664	619 142
				larval area = total water surface area	536 996	8 599 190	128 879	2 063 805
Brielle	28 000	37.0	325.9	larval area = 0.3 water surface	311 136	2 737 812	12 445	109 512
				larval area = total water surface area	1 037 120	9 126 040	41 485	365 042
Poortugaal	54 667	7.4	29.6	larval area = 0.3 water surface	121 525	485 935	9722	38 875
				larval area = total water surface area	405 082	1 619 783	32 407	129 583

On average, the percentage of 4th stage larvae was 30% in Preekhil, 5% in Brielle and 10% in Poortugaal.
Losses from 4th larval stage to adult are estimated at 20%.

vae per m^2 of water surface (Table 1). The estimated densities of larval populations in Preekhil, Brielle, and Poortugaal varied between 161 099–8 599 190; 311 136–9 126 040; and 121 525–1 619 783 per km^2, respectively. Larval densities were considerably higher in Brielle than in the other 2 areas but the mean proportion of 4th larval stages varied considerably between the 3 sites, from 30% in Preekhil, 5% in Brielle, and 10% in Poortugaal. With an estimated survival rate of 80% from fourth larval stage to adult, the daily production of adult mosquitoes per km^2 varied between 9722 in Poortugaal and 2 063 805 in Preekhil (Table 1), depending on inclusion of 30% of the water surface area, or of the entire water surface area. There was no correlation between larval density and chlorinity of the water (Fig. 4).

Figure 4. Relationship between chlorinity of the sampling sites and the number of anopheline larvae collected per dip.



Species Composition and Blood Hosts

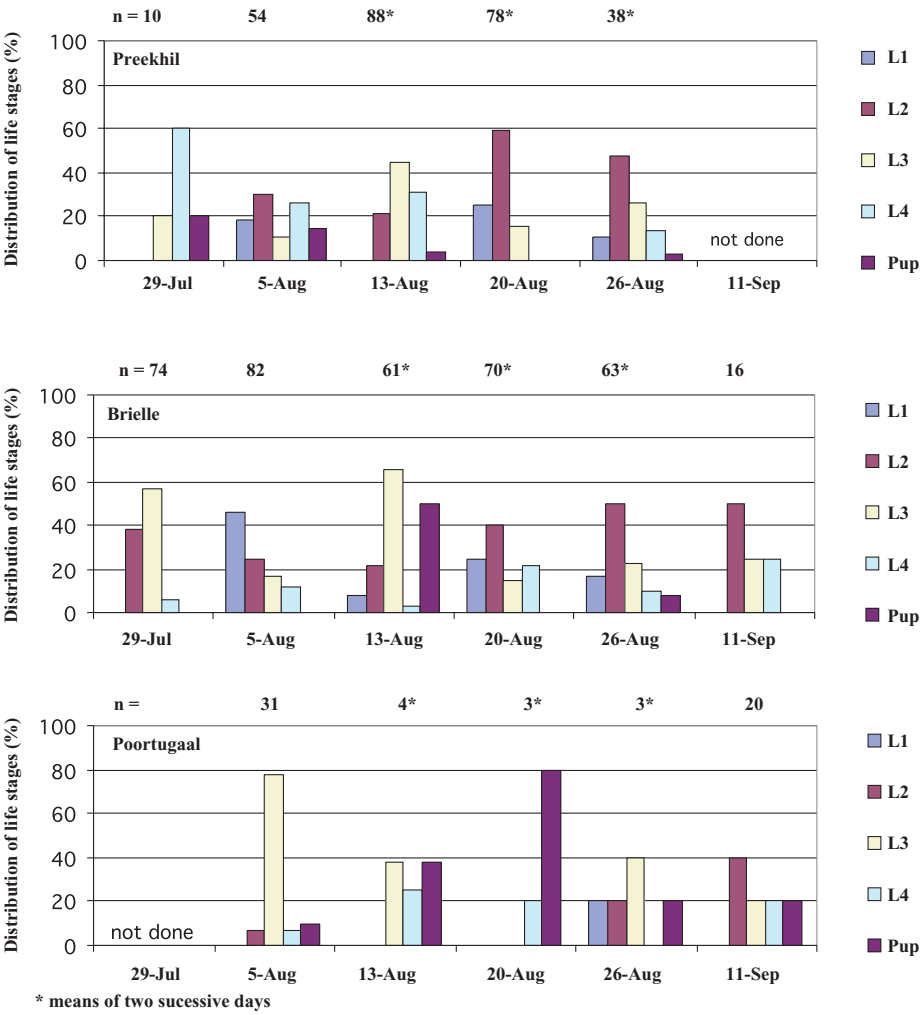
Morphological identification revealed the presence of *A. maculipennis* s.l. and *A. claviger*. 150 larvae of *A. maculipennis* s.l. from 21 sites were identified to species using the PCR technique. Four specimens (2.7%) were *A. atroparvus*, the others *A. messeae*. *A. atroparvus* was found in 3 different sites (Albrandswaard, Beninger Slikken, and Preekhil) (Fig. 1). *A. maculipennis* s.l. that were collected as adult mosquitoes in animal shelters, were identified as *A. messeae*. All females appeared to have recently blood fed, and analyses of 30 blood meals showed that 64% originated from cattle, 28% from horse and 8% from sheep.

Population Dynamics of *Anopheles maculipennis* s.l.

The distribution of larval stages in the 3 sentinel sites from late July until mid-September 1999 is shown in Figure 5. These data are based on the sum of 100 dips per site per sampling day. In some cases they reflect the mean of 2 successive days. In Preekhil the number of larvae reached a peak in mid-August. In contrast, in Brielle the highest larval density was observed on 16th July (440 larvae per 100 dips, data not shown), and from 29th July larval densities remained stable until early September, after which date the numbers decreased rapidly. After an initially high number of larvae in early August in Poortugaal, the density remained low during the rest of August, to rise again in early September. On 20 September, no larvae were found anymore in the three study sites.

All larval stages and pupae were present at most times and there was no clear trend related to cohorts of eggs deposited simultaneously.

Figure 5. Dynamics of larval development of *A. maculipennis* larvae in Preekhil, Brielle, and Poortugaal during the study period. Values above each set of bars represent the (mean) number of larvae collected.



* means of two sucessive days

Vegetation Type and Presence of *Anopheles maculipennis* s.l.

There was a wide variation of floristic and geomorphological characteristics within the sites where larvae of *A. maculipennis* s.l. were found. The most commonly found plant species in sites with a prevalence of *A. maculipennis* s.l. were *Phragmites australis* and *Enteromorpha* sp. followed by *Ceratophyllum demersum* and *Elodea nutalii*. *A. maculipennis* s.l. was also found in ditches overgrown with dense communities of only *Phragmitis australis*.

DISCUSSION

The results show that *A. maculipennis* s.l. is widely distributed in South Holland. All aquatic sites, from ditches and shallow pools in polders to creeks, canals and small lakes on former tidal areas along the Haringvliet may contain larvae of *A. maculipennis* s.l. We did not observe a greater propensity for larvae in rural areas than in areas adjacent to residential sites, such as the town of Brielle. Also, larvae were found in canals and ponds within towns. There was a high propensity for larvae to be present on top of floating vegetation such as *Enteromorpha* and *Ceratophyllum* spp. This is in agreement with previous findings in this area by Van der Torren (2). Surprisingly, we also found many larvae in ditches over-

grown with common reed. To our knowledge, the latter ecosystem has not previously been described as a habitat of *A. maculipennis* s.l. Mud flats that are exposed to tidal fluctuations and which are bordering a fast-flowing river like the Oude Maas (e.g. the nature reserve near Rhoon), cannot be considered larval habitats of this species even though these areas are often densely overgrown with reeds. Even small pools on elevated areas on these mud flats did not contain mosquito larvae. We suggest that these tidal areas are too turbulent for mosquito eggs or larvae, as these are likely to be washed into the sea during infrequent flooding.

As only 2.7% of our larval collections consisted of *A. atroparvus*, we consider most specimens found to be *A. messeae* and the discussion will mostly concern this member of the *A. maculipennis* complex. We found only one published reference on the density and distribution of *A. messeae* in south Holland (2) and, based on this publication and our own observations, we suggest that the current distribution of this species is not very different from that in 1935. Larval densities were also not much different from the 1935 data, and it is possible that *A. messeae* has not undergone a marked ecological change since Van der Torren's study (2). We found the species in many varied habitats, from dominantly reeds (*Phragmites australis*) to floating weeds and algae, to relatively open ditches inside a forest, to clear water in a small lake in the dunes. These latter findings are different from Van der Torren (2), who mentioned that the species is limited to wide ditches with a dense mass of floating vegetation. The near absence of *A. atroparvus* from the study area was surprising, as 60 years ago this species was the most abundant one found on the island of Goeree-Overflakkee. At that time, *A. messeae* was rare on this island. Similarly, *A. atroparvus* was also the most common anopheline of Voorne-Putten island. Indeed, some inhabitants of a retirement home in Rockanje village contracted malaria (*P. vivax*) in 1950 (17), which is further evidence of the historical presence of *A. atroparvus* in this area. The present distribution data of *A. maculipennis* s.l. in the study area suggests that *A. atroparvus* has either lost most of its former habitat to *A. messeae*, or that the former species has become nearly extinct from the area, whereas the latter is still present in its former densities. Our larval collections and subsequent estimates of the population density in Preekhil, Brielle, and Poortugaal, suggest that *A. messeae* does not reach nuisance levels. The species rarely bites man during the summer (5, 18), goes into complete diapause during the fall and winter, and seems to be able to survive on wildlife and domestic grazers. These changes become evident by comparing the 1935 distribution map with that of 1999 (Fig. 6) from which it is apparent that the geographic distribution of *A. atroparvus* has diminished markedly compared to its former distribution. The reasons for the near extinction of *A. atroparvus* may have been the change of the water salinity, pollution of the water by detergents (8) or lack of suitable feeding and resting sites during the winter or possibly all these factors combined. Today, few farmhouses are attached to livestock stables, and the stables are of a much different construction than before, having few dark, sheltered sites where mosquitoes can hibernate.

The results of the larval population studies show that larval densities can increase rapidly during July and August, when the adult mosquito population is in its 3rd generation after the winter diapause. Thus, by mid-July a population of adult mosquitoes has developed and produces large numbers of eggs, which are being deposited in ditches of varying floristic composition. Inspection of half-open livestock sheds in the study area in August 1999 revealed many blood-fed *A. maculipennis* s.l., which were all identified as *A. messeae*. None of the blood meals originated from humans, but were from domestic animals, which corroborates Swellengrebel and De Buck's (5) statement that *A. messeae* is strongly zoophilic and does not feed on humans.

The differences in larval population dynamics between the 3 sentinel sites probably reflect the heterogeneous distribution of the adult mosquitoes, which determines the number of eggs deposited in each site. The presence of first instar larvae as late as 11 September does not necessarily reflect a change in the onset of diapause by day length (2, 19), which is 15 August. Females emerging after that date no longer become reproductively active in that year. However, female mosquitoes that have initiated oogenesis before the time of critical day length may continue producing eggs until death and first instars found in September are likely the offspring of females that emerged in early August.
The large variations in larval densities are probably caused by the environmental differences between the study sites and affected by such variables as vegetation, water quality (12), exposure to direct sunlight and availability of blood hosts. We included the total water surface area as potential larval sites, but this seems unlikely to be the case, and more often around 0.3 of the water surface will reflect the real situation. As a consequence,

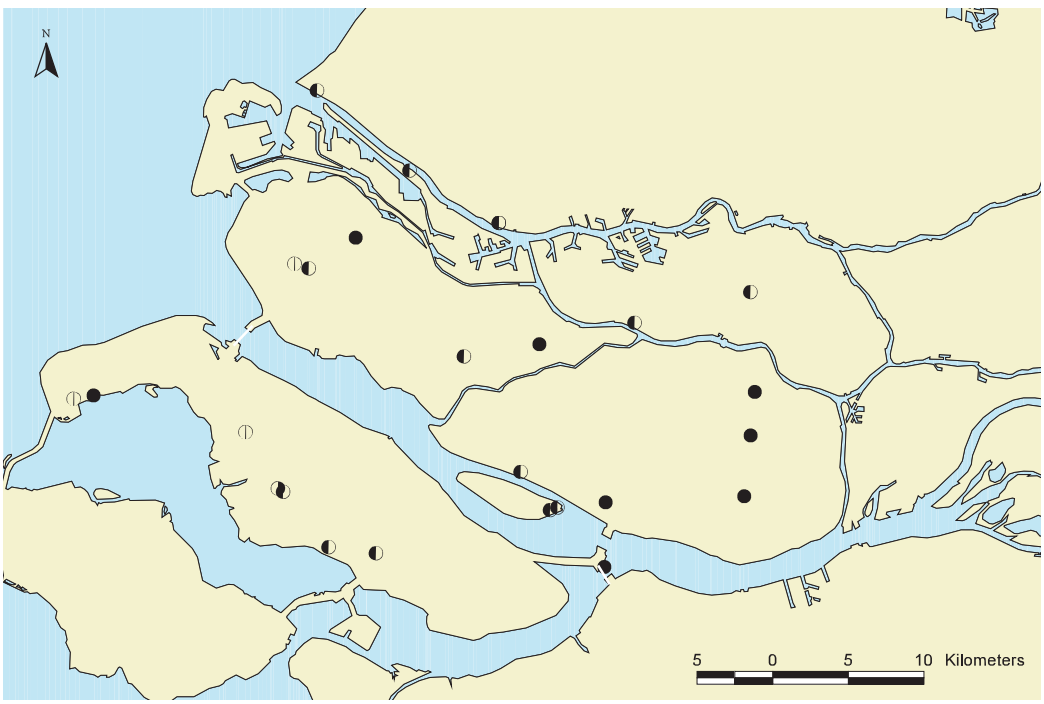


Figure 6. Distribution of *A. maculipennis atroparvus* and *A. messeae* in South Holland in 1933/34 (2) and 1999 (this study). Both halves of the symbol open: no anophelines present; Left-half closed, right-half open: anophelines present in 1933/34, not in 1999: Left-half open, right-half closed; no anophelines in 1933/34, anophelines present in 1999: Both halves closed; anophelines present in 1933/34 and 1999 surveys. Note that in 1999 *A. atroparvus* was only found in Preekhil (2 individuals), Poortugaal (one individual) and Albrandswaard (one individual). (For locations see Fig. 1).

the maximum number of emerging adult mosquitoes km^{-2} day^{-1} cannot exceed 619 142 (Table 1). These numbers could readily find blood on the domestic herds of animals grazing in the study areas. However, as only a small proportion of *A. messeae* will survive the winter diapause (5), mosquito populations must develop from relatively few individuals each year.

Although *A. atroparvus* and *A. messeae* were reported as equally susceptible to *Plasmodium vivax* (20), the high degree of zoophily coupled with the mostly outdoor feeding behavior during the summer, makes the likelihood for local malaria transmission by *A. messeae* remote. In The Netherlands, therefore, *A. messeae* has never been considered a malaria vector and indeed Swellengrebel and De Buck state that the presence of malaria transmission in this country was entirely caused by the blood-feeding and resting behavior of *A. atroparvus* in the fall and winter (5). Elsewhere in Europe (Sweden, Eastern Europe) *A. messeae* has been incriminated as a malaria vector (4, 21). In Germany, coastal malaria was associated with *A. atroparvus*, and inland malaria with *A. messeae* (22). Because the latter reports lack data on the actual infection in the mosquito vectors, it remains difficult to explain why *A. messeae* would have been a vector in Germany, Sweden, and Eastern Europe and not in The Netherlands.

The study shows that in the 65 years since the last ecological survey of anopheline mosquitoes in the province of South Holland, a profound ecological change has occurred, causing the near extinction of *A. atroparvus* in the study area. Since this species is the only mosquito that has been incriminated with historical malaria transmission in The Netherlands, there is no risk for the return of malaria to an endemic state, as has frequently been suggested (23, 24). Although chlorinity levels throughout the study area were reduced compared to historical (1935) levels, they were still sufficiently high to support populations of *A. atroparvus*, should they have been there (2) and this was probably not the reason for the strong reduction of *A. atroparvus*. We suggest that the radical change in opportunities for seeking shelter and blood meals during the fall and winter is the major cause for the recorded low densities of *A. atroparvus*, preventing a population explosion as was common in the pre-World War years in many of the coastal areas of Western Europe. Therefore, it is not expected that the proposed restoration of formerly brackish areas, by re-opening former connections to the North Sea, will lead to a population increase in this malaria vector. *A. messeae* is still widely distributed in the study area, but this species is unlikely to be a malaria vector because in The Netherlands it rarely feeds on man and, unlike *A. atroparvus*, does not blood feed during hibernation. In addition, the natural densities of *A. messeae* are too low to permit transmission of the malaria parasite even were mosquitoes to become infected (Takken and Jetten, unpubl. data).

References and Notes

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25. We are grateful to Stephan Langeweg, Zuiveringschap Zuid Holland, for having provided us with data on the mosquito fauna of South Holland. We thank Dr. Jana Proft for introducing us to the DNA identification methods of *A. maculipennis* and for having provided us with appropriate primers and to Prof. C.F. Curtis and Mr. R. Gill, London School of Hygiene and Tropical Medicine, for having conducted the blood meal analyses. The Swart family in Brielle and Mr. Breen of Ouddorp kindly allowed us to use their garden and grassland. We thank Adrie Jacobs and Bert Heusinkveld, of the Laboratory of Meteorology, Wageningen University, for having provided the meteorological equipment and Theo Ywema and Ronald Gylstra, Laboratory of Aquatic Ecology and Water Quality, Wageningen University for having provided the equipment to record water temperatures. The work of Marjolein Bons and Martin Bubbert, who drew several of the figures, is much appreciated.
26. First submitted 4 Aug. 2000. Accepted for publication after revision 31 May 2001.

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