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IMPACT OF ROCK-POOLS MOSQUITO, *AEDES MARIAE* (CULICIDAE), ON CITIZENS IN A MEDITERRANEAN ISLAND OF SPAIN

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Abstract Nuisances caused by mosquito biting affect the tourism sector, particularly during high season in Mediterranean locations. One species present in these locations is *Aedes mariae* , which breeds in rock-pool coastal zones. There is a lack of information available about the ecology of *Ae. mariae*. The mosquitoes present in the coastal town of Sa Colònia de Sant Jordi (Mallorca, Balearic Islands, Spain) have been studied, comparing the hotel area with the coast area. CDC traps, BG-Sentinel traps, and human landing were used to sample the abundance and behaviour of the culicidae species. Biotic and biochemical data of the rook-pool breeding sites were analysed to understand the oviposition preferences. Adult samplings suggested that *Ae. mariae* has a short host-seeking range, for a short period of time after the sunset they are very aggressive on the rocks, near their breeding spots. Mosquito nuisances in hotels may be mainly related with other *Culex pipiens, Aedes albopictus* or *Aedes caspius*. Water properties in the rook-pools showed that temperature increase the possibility to find *Ae. mariae* larvae and the presence of algae increase their abundance, much more than other factors such as salinity. Control strategies for this species should be focused on their rearing spots.

Key words *Aedes*, mosquitoes, nuisances, tourism, flight range, salinity, control

INTRODUCTION

Mosquitoes are insects belonging to the Culicidae family. Females of these family are haematophagous, which means that they need blood for eggs developing. In Spain are described 65 species of mosquitoes (Robert et al., 2019), being the Aedes albopictus (Skuse, 1894), the Asian tiger mosquito, the most anthropophilic of them (Muñoz et al., 2011). However, other opportunistic species can also bite us while we are close enough to their feeding area. These species usually rear in natural areas (e.g. salt marshes) affecting human settlement included into their flight range. These species usually rear in natural areas affecting human settlement into their flight range.

The relation between humans and mosquitoes has been turbulent since the begging of our species (Becker et al., 2010). Mosquitoes are considered the most deadliest animal in the world by their capacity to transmit pathogens such as malaria, dengue or yellow fever. But they also reduce our quality of life with their bites, affecting the tourism industry (UNDP 2017). In France the presence of mosquitoes in the Mediterranean coast stopped the tourism development, so government agency was created to control mosquitoes: the Interdepartmental agreement for Mosquito Control (EID).

In the current work we have studied the mosquito problems registered in Sa Colonia de Sant Jordi, a coastal town located in the South of Majorca island (Balearic Islands, Spain). This location is an important touristic destination with 4.000 hotel beds, with one of the most visited beaches in Mallorca. It is also an interesting location for mosquito control, since mosquito nuisances are a recurrent problem during summer seasons, reducing the aforementioned quality of tourism and neighbourhood lifestyle. These nuisances are caused by 4 different species in theory. *Aedes caspius* can rear in nearby wetlands and marshlands, producing high population densities when the phreatic surface increases. Single-family houses with unattended swimming pools can also turn into spots for *Culex pipiens*. Since 2016 *Ae. albopictus* is present in this town, with a population increasing year after year. And rock-pools from the rocky coast are the perfect environment for *Aedes mariae*.

The aim of this research is to determine the pressure exerted by different mosquito species on the hotel area of Sa Colonia de Sant Jordi. We will focus on *Ae. mariae*, a halophile specie distributed along the occidental coast of the Mediterranean Sea. *Ae. mariae* is not a vector of human diseases (Ribeiro et al 1988), but it can transmit the parasite *Plasmodium relictum*, that cause malaria in birds (Gutsevich et al. 1974). Margalef (1949) studied its biology, however, studies about this species are currently scarce. In this study, we recorded larval habitat characteristics and hosting behaviour of this species.

MATERIALS AND METHODS

Two sampling stations were stablished: one in the coast rocks near the breeding sites of Ae. mariae, 12,5 m away from the sea, called “Rocks” hereafter, and the other one near bushes in a seafront hotel, 70 m away from the sea, called “Hotel” hereafter. There are 300 meters between these two stations. One BG-Sentinel-2 trap with BG-Lure and CO_2, and one CDC trap with CO_2 were placed into each sampling station. In Rocks station, Human Landing captures (HLC) were also recorded. Captured mosquitoes were identified according to Becker (et al., 2010). Five samples were conducted monthly from June until October (May 28, June 29, August 30, September 29 and October 29). Traps were placed 2 hours (+/- 30 minutes) before sunset, and retired 1 hours after, remaining 3:30 hours (+/- 30 minutes) in the field. The HLC were carried out by 2 people, which they were sampling mosquitoes each other with a mouth aspirator, since 20 minutes before the sunset till 1 hour after, remaining 1:20 minutes exposed in the field. The time of capture (minutes after the sunset) for each mosquito was recorded.

A randomly chosen number of rock-pools were sampled near the “Rocks” station. We determined the number of *Ae. mariae* larvae, biotic factors (algae cover (0: No algae – 4: all coverd), presence of other fauna (Yes/No) and *Posidonia oceanica* (L.) leaves (Yes/No)) and physicochemical values (pH, temperature, TDS, conductivity and salinity). A multiparametric PC-5 tester (XS Instruments, Italy) was used to determine the physicochemical values. The rock-pools were randomize sampled, from March 22 until June 07 (8 times).

Table 1. Total Number of mosquitoes captured in traps.

	Rocks		Hotel	
Mosquito	**CDC trap**	**BG Sentinel trap**	**CDC trap**	**BG Sentinel trap**
Cx.pipiens		4		16
Ae.albopictus				15
Ae.caspius			6	6
Ae.mariae	1	4		1
Ae.detritus				1
Cq.richiardii		1		

The potential effect of physicochemical parameters of the rock-pools were statistically analysed to determine the importance of each factor to explain the presence or absence of larvae. This analysis was carried out using a generalized linear model GLM with a binomial distribution (logit link function). Lower values of the Akaike’s information criterion (AIC; Burnham and Anderson, 2002) were used to select the best fitting model. To determine abundance of larvae into each rock-pool a GLM analysis was used with a Poisson distribution (log link function), where number of larvae were compared with the physicochemical parameters of the rock-pools and biotic factors. The multimodel inference analyses were performed using the ‘MuMIn’ package (Barton and Barton 2019) implemented in R version 3.6.0 (R Development Core Team 2017).

RESULTS AND DISCUSSION

Adult traps captured 55 mosquitoes from 7 different species (Table 1) along all the sampling period. The “Hotel” station captured 3.5 times more mosquitoes than Rocks station. In fact, the “Hotel” station captured the highest number of species, mainly *Cx. pipiens, Ae. albopictus and Ae. caspius*. Conversely, on the Rocks station we captured mainly *Ae. mariae* and *Cx. pipiens*. This means that into the hotels area of Sa Colonia de Sant Jordi there were more mosquitoes than in the coast side, with the presence of *Ae. albopictus*, a diurnal mosquito very aggressive for humans.

Table 2. Significance of models to explain presence/absence of *Ae. mariae* in rock-pools.

MODEL	df	Deviance	AIC
Model 1: Presence ~ 1	0	114,573	116,57255
Model 2: Presence ~ pH	1	111,461	115,46141
Model 3: Presence ~ Conductivity	1	110,009	114,00942
Model 4: Presence ~ Salinity	1	113,305	117,30455
Model 5: Presence ~ TDS	1	109,235	113,235
Model 6: Presence ~ Temperature	1	76,916	80,91628

We found differences between the 3 adult sampling methods. BG Sentinel method captured individuals of all the different species, but HLC method captured more *Ae. mariae*. In September no captures were registered in HLC because weather was not favourable for mosquito flight. There are no statistical differences between the CDC traps from both stations, but it may be due the few mosquitoes captured by this trap.

Ae. mariae activity is gathered along few minutes after the sunset (Figure 1). Into the first HLC sampling on June 28, females were active over 26 minutes, while in October 29 they remain active over 10 minutes. This reduction could be caused by the sunset speed in the autumn months. In fact, there are significant differences between the timing of *Ae. mariae* activity, showing a reduction from 17 minutes after the sunset in June to 9 minutes in October.

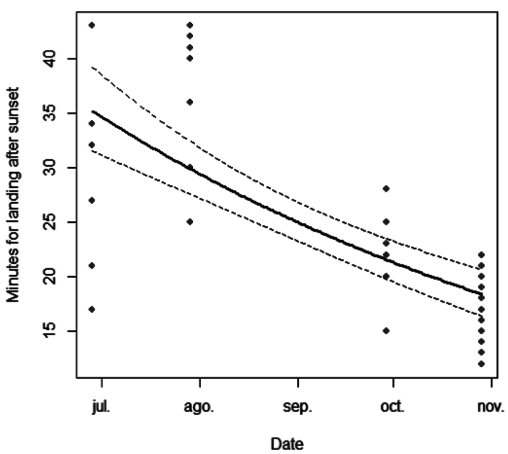

Figure 1. Number of *Ae. mariae* individuals collected with Human landing captures. Each dot is a mosquito, and time is referred to the sunset of that day.

The physicochemical characteristics of conductivity, salinity and TDS in the rocks-pools water were highly correlated since they measure similar properties. Conductivity was the variable that better fitted to the data, so we decided to discard the other variables in posterior analysis. Temperature was the variable that more affected the presence of larvae (Table 2). The seasonal activity from March to October (Becker et al., 2010), could explain this relationship; since the rock-pools sampling take place in spring while the *Ae. mariae* population is increasing.

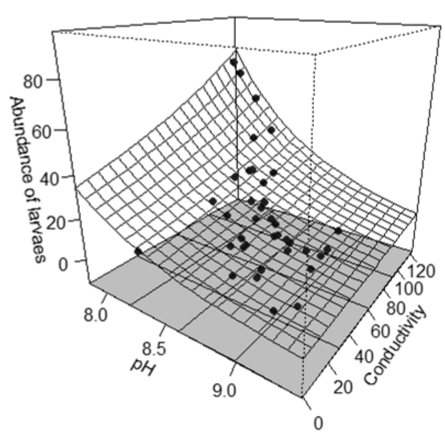

Figure 2. The pH and conductivity linked to the abundance of *Ae. mariae* larvae.

The pH and conductivity were the abiotic factors that explained better the abundance of *Ae. mariae* larvae in the rock-pools (Figure 2), while all the biotic factors tested were extremely important for the abundance of larvae, mainly the algae cover (Figure 3a). Margalef (1949) studied the ecological limitations of this species and found that the absence of algae in the seawater is a limiting factor, similar to the results of the present study. Margalef (1949) found a possible predation competence, and we determine the presence of other fauna had a negative effect on the abundance of *Ae. mariae* larvae, not necessarily in a predatory relationship. Rioux (1958) found larvae in rock-pools with salinity up to 200g/l. Salinity had a wide range valies in the rock-pools with larvae, from 1g/L until 77,4g/L. Seawater has 37,5 g/L of salt, so there are rock-pools filled with rainwater (20 parts per million), others with seawater and others mixed, highlighting the adaptive capacity of *Ae. mariae* larvae.

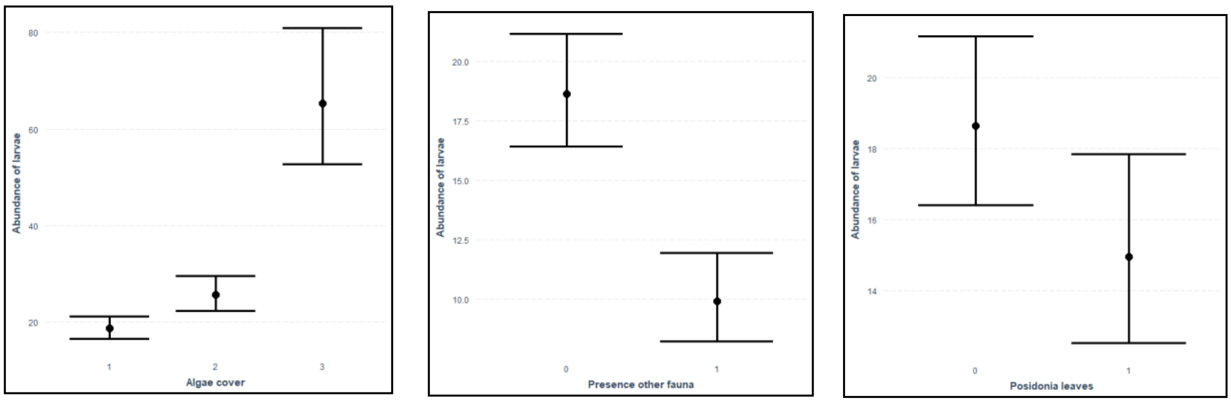

Figure 3. Predicted values of different biotic factors: a: Algae cover; b: Presence of other fauna; c: Posidonia leaves) over the abundance of *Ae. mariae* larvae in the rock-pools.

CONCLUSIONS

There are several species of mosquitoes affecting Sa Colonia de Sant Jordi, but they distribution differs between the hotel area and the coast. *Cx. pipiens*, *Ae. albopictus* and *Ae. caspius* were the most abundant mosquitoes in the hotel area. Each species has its own biological characteristics, so the control methods should be focus on their biology. The integrated mosquito control is the best approach to reduce the nuisances to the tourists and citizens, and the reduction of their rearing spots should be prioritised. CDC and BG-Sentinel traps did not provide a reliable information of *Ae. mariae* bite pressure, HLC the best method to determine it. The effort to use HLC was justified due to the number of mosquitoes captured and the information obtained. The short slot of time when this species is searching for hosts is about 15 minutes after the sunset along 10 to 26 minutes.

Ae. mariae is mainly present in the coast because it usually breeds in hypersaline rock-pools. Salinity of the rearing spots is a positive variable, which increase the probability to find larvae in the rock-pools. There is an absence of larvae when the salt solidify in the surface of the rock-pool, so we hypothesise that this layer prevents larvae from breathing. Presence of algae in rock-pools was the factor that favoured more the abundance of larvae, since larvae feed on these.

Control methods for *Ae. mariae* should be focussed on the rock-pools, detecting them, planning the treatments according with the tides, waves or precipitation, and finally treating them with *Bacillus* based formulations. Other control methods like physical barriers (polydimethylsiloxane, perlite) or fill these pools with expanding foam like in tree holes (CDC 2016) are not recommended because this is a natural scenario, commonly affected by the waves. In our study, we have found 3 to 17 rock-pools with larvae in about 25.000 sq.m. which was a number that can be easily treated by a pest control company.

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