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Ecology and diversity of mosquito larvae in ponds and lagoons of Northwestern Spain

Ecología y diversidad de las larvas de mosquitos en charcas y lagunas del noroeste de España

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Abstract

Galicia, located in the northwestern part of Spain, has a great number and variety of aquatic ecosystems where mosquitoes can breed. Despite the sanitary relevance of these insects, studies on mosquito populations in the region are still scarce. The field research was carried out in 48 sampling points (27 continental lagoons, 12 coastal lagoons and 9 temporary ponds) throughout the entire Galician territory. The samples were collected intermittently and seasonally through different water quality monitoring projects between 2001 and 2017. More than 1500 mosquito larvae belonging to 10 species of five genera (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex* and *Culiseta*) were identified. *Anopheles (Anopheles) maculipennis* s.l. Meigen was the most widely distributed species in the study, being especially dominant in rural areas. In contrast, *Culex (Culex) pipiens* Linnaeus and *Culex (Culex) theileri* Theobald showed a preference for breeding in urban areas. New contributions to the knowledge about the larval ecology and distribution of these mosquito species are made throughout this study, including information about the tolerance of each species to water parameters (temperature, pH, dissolved oxygen and conductivity). Likewise, the relationship between culicid diversity and the habitat characteristics of the breeding sites (water body type, climate and level of anthropization) is discussed.

KEYWORDS
Anopheles, *Coquillettidia*, *Culex*, *Culiseta*, Galicia, vector

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are closely linked to wetlands, as water is an essential requirement for larval and pupal development. The study of these insects is not only important for ecological reasons but also for sanitary motives as they are important vectors of human and other animal diseases. Reducing the health risk associated with mosquitoes is more effective when control measures are applied to the larval stages as it is the time when individuals are spatially concentrated. In addition, this prevents biting by adults and avoids the use of adulticids and non-selective treatments. Therefore, detailed local studies on mosquito larval habitats are essential to apply adequate management measures.

The Autonomous Community of Galicia, in northwestern (NW) Spain, has a large number and diverse freshwater ecosystems (Benetti et al., 2012) where mosquitoes can breed. Recently, the increasing risks associated with vector-borne diseases have led to the implementation of West Nile Virus (WNV) and bluetongue surveillance programmes in Galicia, which have improved the knowledge on the diversity and distribution of mosquitoes (Lucientes et al., 2012; Lucientes & Delacour-Estrella, 2012; Martínez-Barciela et al., 2020; Polina et al., 2022). However, most of these efforts have been focused on the use of traps and the capture of adults in areas where hosts are present or where there is a risk of entry of invasive species (livestock farms, airports, ports, industrial states, etc.), neglecting the monitoring of natural larval habitats such as wetlands.

Although numerous studies on water quality and macroinvertebrate biodiversity in Galician wetlands have been carried out over the years, mosquitoes have been overlooked in favour of other taxonomic groups of greater conservation interest (Álvarez-Troncoso et al., 2014; Benetti et al., 2007; Pérez-Bilbao et al., 2010). The aim of this work is to extend the knowledge of the culicids of Galicia through a detailed study of larval populations in its wetlands. The relationship between culicid diversity and the habitat characteristics of the breeding sites (water body type, climate and level of anthropization) is also analysed. The results of this research may be of great interest to expand the available information about the ecology (preference for breeding sites and adaptation to different water parameters and habitat characteristics) of the studied mosquito species.

MATERIALS AND METHODS

The field research was conducted in 27 continental lagoons, 12 coastal lagoons and 9 temporary ponds throughout the Autonomous Community of Galicia (NW Spain) (Figure 1). All sampling points that were geographically close and with a common environmental feature (climate, flora, fauna, etc.) are considered within the same area and named accordingly (Table 1). A total of 35 areas were sampled, with 27 of them considered as Special Areas of Conservation (SAC) under the European Union Habitats Directive (Natura 2000 Network) standard (Table 1). All sampling points fit into two types of temperate climate according to the Köppen–Geiger climate classification: temperate oceanic climate (Cfb) and warm-summer Mediterranean climate (Csb), which correspond to the two most representative climates of Galicia (Figure 1). The first has cold winters and cool summers with uniform rainfall between seasons, while the second has cold or mild winters and dry and cool summers, with irregular rainfall throughout the year. The study area also was classified into three degrees based on its level of anthropization: high (H) (urban or peri-urban areas with human activity within a radius of 500 m), medium (M) (rural areas with human activity within a radius of between 500 m and 1 km) and low (L) (natural areas without human activity within a radius of 1 km) (Table 1). Generally, the sampling points are characterized by abundant vegetation in the surroundings (Figure 2),

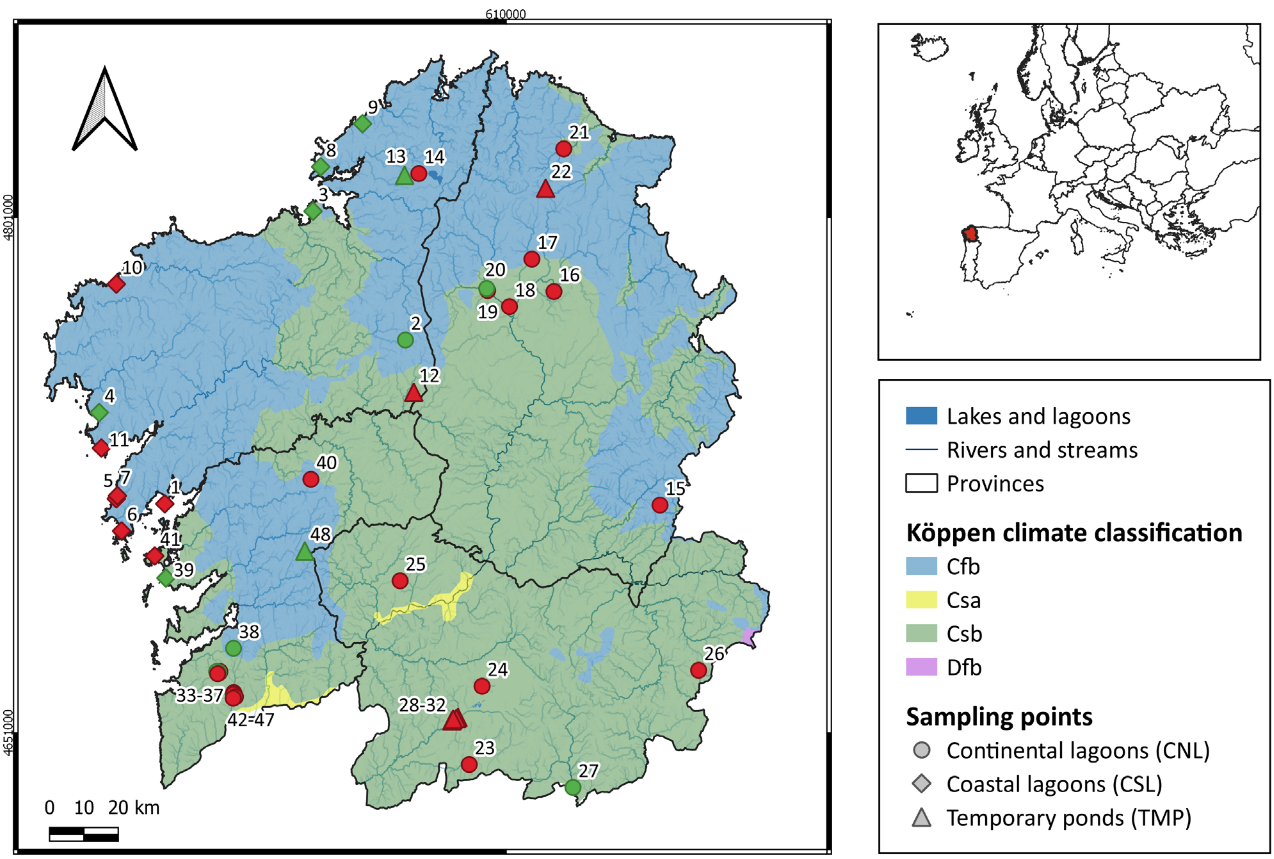

FIGURE 1 Presence (red) and absence (green) of mosquitoes in the selected sampling points (numbers refer to the sampling points' codes indicated in Table 1) (Galicia, Spain). Map of the Köppen climate classification extracted from the Instito Geográfico Nacional (IGN España).

TABLE 1 Sampling point information, including the code number (N°), province (P) (C: A Coruña, L: Lugo, O: Ourense, P: Pontevedra), sampling point/place's name, UTM coordinates (Datum WGS 84), altitude in metres (Alt.), water body type (W) (CSL: coastal lagoon, CNL: continental lagoon, TMP: temporary pond), level of anthropization (LA) (H: high, M: medium, L: low), sampling season (A: autumn, M: summer, S: spring, W: winter), sampling year and number of samplings (*n*).

Code	P	Name	UTM (29T)	Alt.	W	LA	Season	Year	*n*
1	C	Carragueiros[a]	510,609,4,717,695	9	CSL	H	M	2010	1
2	C	Sobrado dos Monxes[a]	580,663,4,765,357	523	CNL	M	M	2010	1
3	C	Mera[a]	553,765,4,802,827	13	CSL	H	M	2010	1
4	C	Carnota	491,498,4,744,243	24	CSL	M	M	2007	1
5	C	Muro	496,492,4,719,152	11	CSL	M	S	2007	1
6	C	Vixán	498,024,4,709,790	7	CSL	H	S,M	2007–08	4
7	C	Xuño	496,818,4,720,059	14	CSL	M	S,M	2007	2
8	C	Doniños	555,987,4,815,762	−5	CSL	H	S,M	2007–08, 2017	5
9	C	Valdoviño	568,175,4,828,290	0	CSL	H	S	2007–08	2
10	C	Traba	496,494,4,781,619	2	CSL	H	S,M	2007–08, 2017	5
11	C	Louro	492,142,4,733,921	13	CSL	H	S,M	2007–09, 2017	6
12	C	Gándaras de Melide	583,088,4,750,166	452	TMP	H	S,M	2007–09, 2017	6
13	C	A Capela	580,417,4,813,310	524	TMP	L	S	2008	1
14	C	As Pontes	584,566,4,813,790	432	CNL	H	S	2007–08	2
15	L	Lucenza	654,786,4,717,304	1380	CNL	L	S,M	2007–09, 2010	6
16	L	Caque	623,915,4,779,463	425	CNL	H	S,M	2007–08, 2017	3
17	L	Cospeito	617,487,4,788,900	407	CNL	H	S,M	2007–11, 2017	8
18	L	Lagoa do Rei	610,965,4,775,074	418	CNL	H	S,M	2007–08	4
19	L	Pozo do Ollo	604,596,4,779,768	422	CNL	M	W,S	2007–08	2
20	L	Rio Caldo	604,323,4,780,357	422	CNL	M	S	2007–08	2
21	L	Ouro	626,769,4,820,985	62	CNL	M	S,M	2007–08	2
22	L	Abadín	621,488,4,809,544	636	TMP	L	S,M	2007–09, 2017	6
23	O	Tosende[a]	599,245,4,641,827	868	CNL	L	S,M	2007–08	4
24	O	Piñeira de Arcos[a]	602,984,4,664,622	630	CNL	H	M	2010	1
25	O	Puzo do Lago[a]	579,120,4,695,292	392	CNL	M	M	2011	1
26	O	Pradorramisquedo	666,034,4,669,252	1452	CNL	L	S	2007–08	2
27	O	Támega	629,455,4,635,139	377	CNL	H	S	2007	1
28	O	Veiga de Ponteliñares 1	595,969,4,655,506	625	TMP	M	W,S,M	2001–02	12
29	O	Veiga de Ponteliñares 2	594,191,4,654,543	621	TMP	M	W,S,M	2001–02	12
30	O	Veiga de Ponteliñares 3	595,536,4,655,284	620	TMP	M	S	2007–08	2
31	O	Veiga de Ponteliñares 4	594,541,4,654,951	621	TMP	M	W,S,M	2001–02	12
32	O	Veiga de Ponteliñares 5	594,588,4,655,028	625	TMP	M	S,M	2007–09	13
33	P	Lagoas Marcosende 1[a]	526,645,4,668,889	256	CNL	H	W,S,M,A	2001	4
34	P	Lagoas Marcosende 2[a]	526,487,4,668,543	256	CNL	H	W,S,M,A	2001	4
35	P	Lagoas Marcosende 3[a]	526,328,4,668,710	256	CNL	H	W,S,M,A	2001	4
36	P	Lagoas Marcosende 4[a]	525,712,4,668,881	256	CNL	H	W,S,M,A	2001	4
37	P	Lagoas Marcosende 5[a]	525,994,4,668,242	409	CNL	H	W,S,M,A	2001	4
38	P	Mol[a]	530,616,4,675,635	254	CNL	H	M	2011	1
39	P	Praia Maior[a]	510,937,4,696,084	13	CSL	H	M	2011	1
40	P	Sacra de Olives	553,148,4,724,818	678	CNL	L	W,S,M,A	2007–08, 2010–11	11
41	P	Bodeira	507,666,4,702,502	11	CSL	H	S,M	2007–08, 2010–11, 2017	7
42	P	Gándaras de Budiño 1	530,719,4,662,466	22	CNL	H	W,S,M,A	2003–05	17
43	P	Gándaras de Budiño 2	530,669,4,662,775	26	CNL	H	W,S,M,A	2003–05	17

(Continues)

TABLE 1 (Continued)

Code	P	Name	UTM (29T)	Alt.	W	LA	Season	Year	*n*
44	P	Gándaras de Budiño 3	530,812,4,662,430	10	CNL	H	W,S,M,A	2003	4
45	P	Gándaras de Budiño 4	530,588,4,662,271	28	CNL	H	S	2007–08	2
46	P	Gándaras de Budiño 5	531,169,4,661,536	25	CNL	H	W,S,M,A	2003–05	16
47	P	Gándaras de Budiño 6	530,534,4,661,108	23	CNL	H	W,S,M,A	2003–05	16
48	P	Serra do Cando	551,385,4,703,974	942	TMP	L	S	2007–08	2

[a]Not Special Area of Conservation (SAC).

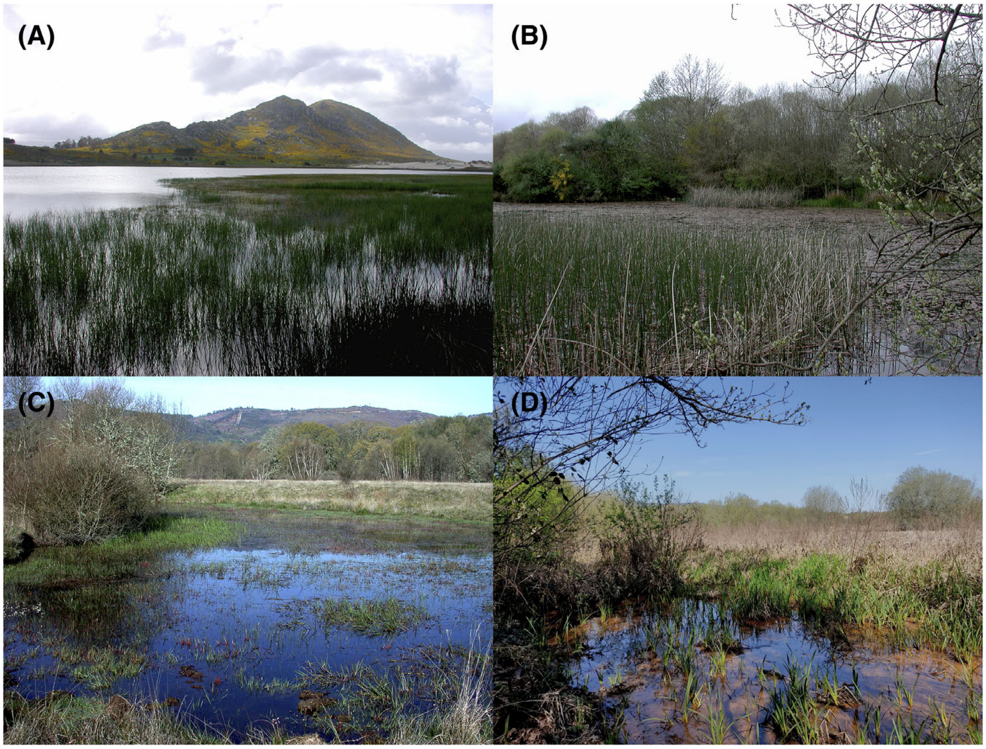

FIGURE 2 Photographs showing typical breeding sites where sampling was carried out: coastal lagoon of Louro (a), continental lagoon of Cospeito (b), temporary pond of Veiga de Ponteliñares 5 (c) and continental lagoon of Gándaras de Budiño 1 (d).

with aquatic plants such as *Phragmites* sp. (Poaceae) and *Juncus* sp. (Juncaceae) being particularly dominant, as well as riverside trees such as *Populus* sp. and *Salix* sp. (Salicaceae).

The sampling was carried out intermittently and seasonally through different projects focused on water quality assessment and aquatic macroinvertebrate biodiversity between 2001 and 2017 (Table 1). Most of the sampling occurred in spring (mid-April to early June) and summer (late June to mid-September), complemented by occasional monitoring in autumn (late September to early December) and winter (late December to mid-March). Fauna was collected intensively stirring the substrate with an entomological net (500 μm mesh, 30 cm diameter and 60 cm deep) covering all different types of habitats present at each sampling point. Physical-chemical data (temperature, pH, dissolved oxygen and conductivity) were measured in situ using a multiparameter water quality meter (HI 98194-HANNA Instruments) (Leighton Buzzard, Bedfordshire, England). The probe was introduced into the water at the time of sampling and removed once all the parameters were stabilized and could be recorded. The material was transported to the laboratory in 99% ethanol. Larvae belonging to the Culicidae family (4th instar larvae) were placed in Petri dishes with 70% ethanol and analysed under binocular magnifier for identification to the species level according to Becker et al. (2020). The specimens were conserved in 70% ethanol and deposited in the scientific collection of the Aquatic Entomology Laboratory at the University of Vigo (Vigo, Galicia, Spain).

Species richness (S), Shannon–Wiener (H′) and Simpson's (DS) indexes were applied to determine the diversity and dominance of culicids in the study area considering the water body type, climate and level of anthropization. Since these metrics did not follow a normal distribution, the Kruskal–Wallis test was applied to determine statistical differences between groups. The data analysis was run

TABLE 2 Sampling and ecological data by species, including number of collected mosquito specimens (N), mosquito frequency percentage (F) [(samples with mosquitoes/total samples) × 100], mosquito distribution percentage (D) [(areas with mosquitoes/total areas) × 100], altitude range (Alt.), temperature range (Tª), pH range (pH), dissolved oxygen range (OD) and conductivity range (CE).

Species	N	F (%)	D (%)	Alt. (m)	Tª (°C)	pH	OD (mg/l)	CE (μS/cm)
Aedes caspius	1	0.4	2.9	11	26	8.72	11.8	7.65
Anopheles maculipennis s.l.	643	21.6	42.9	7–868	10.5–29.1	4.1–9.6	1.2–8.9	1.5–1408
Coquillettidia buxtoni	15	3.7	11.4	7–407	7.7–23.4	5.8–7.2	1.3–11.2	51.2–1408
Culex impudicus	10	1.2	8.6	11–868	22.4–27.8	5.8–8.2	7.2–7.9	42.8–1250
Culex pipiens	110	4.1	20	7–868	16.1–27.2	5.8–8.7	0.6–14.3	7–1408
Culex theileri	52	4.1	20	7–678	16.6–29.1	6.2–9.6	3.5–10.1	11.4–1408
Culex territans	39	2.9	14.3	10–868	14.2–22.4	5.8–7.7	0.6–8.8	31.4–740
Culiseta annulata	44	2	11.4	11–28	15.2–27.2	6.4–8.3	3.5–16.5	7–291
Culiseta fumipennis	536	5.3	11.4	452–1452	1.6–18.4	5.9–7.5	5.4–20.7	8.6–95.5
Culiseta morsitans	1	0.4	2.9	10	12.1	5.1	2.81	71.7

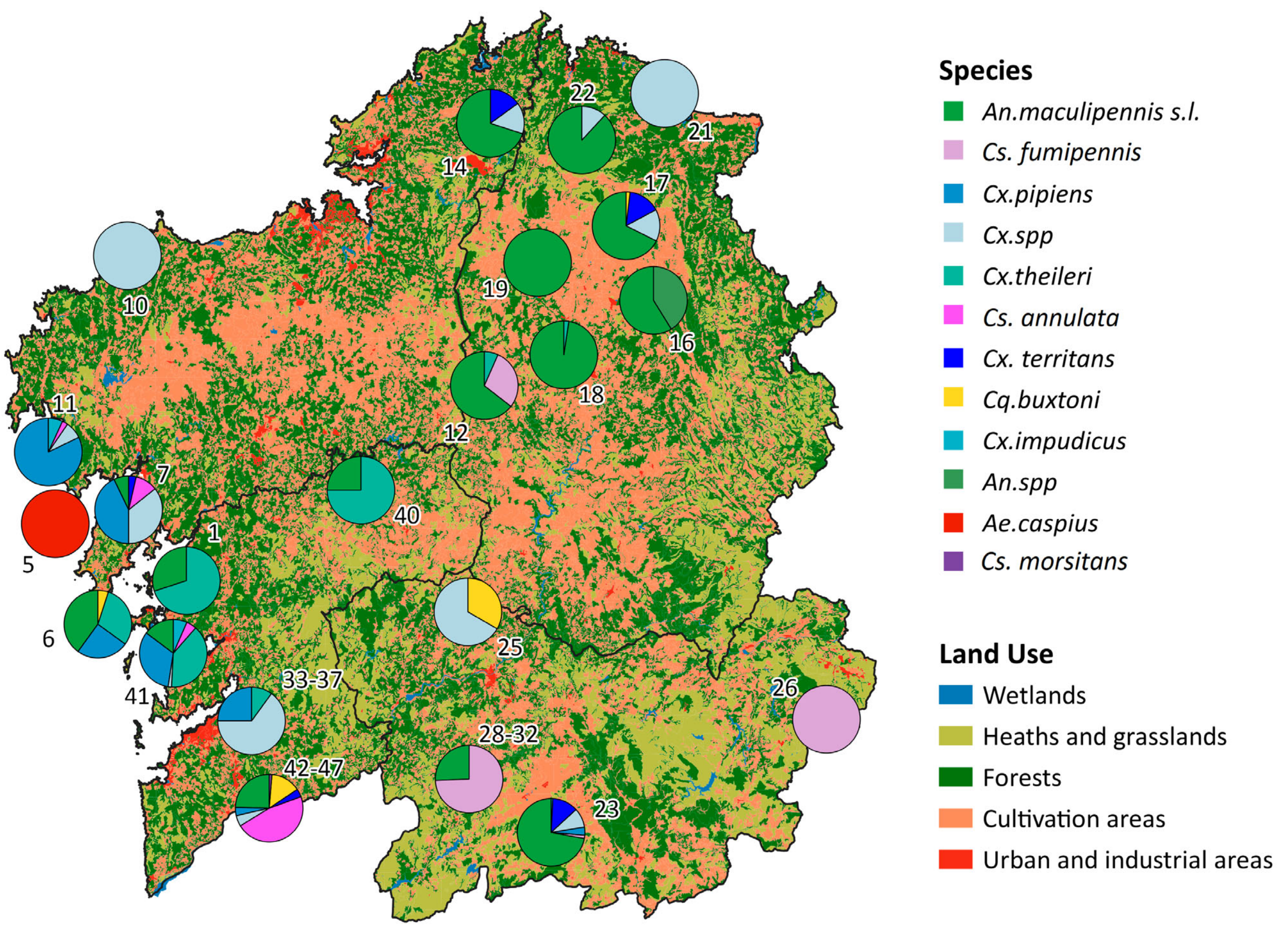

FIGURE 3 Distribution and proportion of mosquito species (*Ae.*: *Aedes*, *An.*: *Anopheles*, *Cq.*: *Coquillettidia*, *Cx.*: *Culex*, *Cs.*: *Culiseta*) according to the sampling points (numbers refer to the sampling points' codes indicated in Table 1) (Galicia, Spain). Land use map (CORINE 2006) extracted and modified from Instituto Geográfico Nacional de España—Xunta de Galicia.

using the BiodiversityR package of version 4.2.0 of the R software (Kindt & Coe, 2005). The characterization of the study area and the development of maps were applied using version 3.8 of Quantum GIS (QGIS) (QGIS Development Team, 2009).

RESULTS

A total of 246 samples and 1535 larvae were analysed (Table 1), with 84 of them identified only at genus level due to their poor morphological condition (seven individuals as *Anopheles* spp. and 77 individuals as *Culex* spp.). Among the 1451 remaining larvae, 10 species belonging to five genera were confirmed and relevant data about their sampling and ecology were recorded (Table 2). *Anopheles maculipennis* s.l. Meigen was found to be the most abundant, most commonly found and widely distributed mosquito in the study area. *Culiseta fumipennis* (Stephens) was the second most abundant species, while *Culex pipiens* Linnaeus and *Culex theileri* Theobald had a wider range of distribution. *Culiseta annulata* (Schrank) also is associated to anthropized areas, but it was only found at low altitudes. *Culex territans* Walker, *Coquillettidia buxtoni* (Edwards) and *Culex impudicus* Ficalbi were detected less frequently, showing tolerance for breeding in saline or polluted waters. *Aedes caspius* (Pallas) and *Culiseta morsitans* (Theobald) were captured occasionally, both in lagoons at low altitudes.

TABLE 3 Abundance (N), species richness (S), Shannon–Wiener (H′) (H′ < 2 = low values) and Simpons's (DS) (DS < 0.5 = low values) indexes pooled by continental lagoons (CNL), coastal lagoons (CSL) and temporary ponds (TMP); temperate oceanic climate (Cfb) and warm-summer Mediterranean climate (Csb); and high level of anthropization (H), medium (M) and low (L).

	N	S	H′	DS
CNL	491	9	0.98	0.43
CSL	194	8	1.30	0.63
TMP	850	3	0.70	0.47
Cfb	216	8	1.27	0.65
Csb	1319	9	1.20	0.62
H	609	9	1.50	0.67
M	193	7	0.73	0.44
L	733	6	0.64	0.29

TABLE 4 Results of the Kruskal–Wallis test (H: test statistics, df: degree of freedom, *p*: *p*-value) for each index (N: abundance, S: species richness, H′: Shannon–Wiener Diversity Index, DS: Simpson Index) by habitat characteristic.

		H	df	*p*
Water body type	**N**	**5.33**	**2**	**0.07**
	S	0.72	2	0.70
	H′	1.44	2	0.49
	DS	3.69	2	0.16
Köppen climate	**N**	236	1	0.12
	S	0.59	1	0.44
	H′	0.01	1	0.89
	DS	**3.35**	**1**	**0.07**
Level of anthropization	**N**	0.51	2	0.77
	S	0.06	2	0.98
	H′	0.54	2	0.76
	DS	1.71	2	0.43

Note: Statistical differences at a significance level 0.1 are indicated in bold.

Culicids were detected in most of the sampling points (73%) (Figure 1), but in three of them (Lucenza, Piñeira de Arcos and Lagoa Marcosende 2) species could not be determined due to larvae immaturity. In total, culicids were successfully identified in 32 sampling points and 22 areas, in each of which particular proportions of species were recorded (Figure 3). The presence of larvae was detected with the same relative frequency in continental lagoons and temporary ponds (78%), being lower in coastal lagoons (58%). The Csb climate recorded a higher frequency of mosquitoes (81%) compared to the Cfb climate (59%), as did areas with a medium level of anthropization (77%) compared to those with high and low anthropization (72%). The breeding sites with mosquitoes encompassed a wide range of water parameters. The average temperature was 18.9 ± 5.3°C, the pH was 6.8 ± 1, the dissolved oxygen was 7 ± 4.2 and the conductivity was 187.1 ± 279.4.

The metrics calculated (N, S, H′ and DS) for each group of habitat characteristics (water body type, climate and level of anthropization) are variable (Table 3). Statistical differences were detected at a significance level 0.1 in abundance between water body types, as well as in Simpson's Index between Köppen climates. However, no significant differences were detected at a significance level 0.05 (Kruskal–Wallis test: *p*-value >0.05) (Table 4).

DISCUSSION

Anopheles maculipennis s.l. is widely distributed in the study area, being especially dominant in the rural areas of the province of Lugo (northeast of Galicia) (Figure 3), from less than 10 m to more than 800 m in altitude. It is a complex of species of known Palearctic distribution that is present in most European countries and well distributed in Spain (Bueno-Marí et al., 2012). So far, its presence has been registered in three provinces of Galicia (Polina et al., 2022), but now its detection in A Coruña extends its known distribution to the four Galician provinces. Although it has been documented that *Anopheles* mosquitoes prefer to breed in fresh and clean waters (Manguin, 2013), the obtained results demonstrate their tolerance to wide ranges of temperature, pH and conductivity (Table 2). This confirms the previous studies of Emidi et al. (2017) and Amawulu et al. (2020), which found that these mosquitoes breed in a great diversity of habitats: from fresh waters to polluted ones, including those with high saline inputs such as coastal lagoons. Likewise, the detection of these larvae in waters close to pH 4 shows that mosquito mortality does not necessarily occur below pH 4.5 as suggested by Tiimub et al. (2012). Larvae were found from April to November and occasionally sharing breeding site with those of *Cx. theileri*, *Cx. territans*, *Cs. fumipennis*, *Cq. buxtoni*, *Cx. pipiens*, *Cx. impudicus* and *Cs. annulata*. The *An. maculipennis* complex comprises several sibling species that differ in their breeding, feeding and epidemiology, including the historically major malaria vector of Spain *Anopheles atroparvus* Van Thiel (Delacour-Estrella et al., 2012).

Culiseta fumipennis is widely distributed throughout Europe (Becker et al., 2020) and to a lesser extent in Spain (Bueno-Marí et al., 2012; González et al., 2020). In Galicia it has only been detected in four areas in the centre and south of the region, being only present in the provinces of Lugo and Ourense (Figure 3). Even so, it is the second most abundant mosquito in the study area (Table 2), as it has been found breeding in high densities, especially in spring. This species is found frequently in mountainous environments (Bueno-Marí et al., 2009) and can be found in temporary ponds with rich vegetation (Becker et al., 2020), which is consistent with this study. Larvae have always been found above 400 m altitude, in well-oxygenated waters and at low temperatures (even below 2°C) (Table 2), showing a tendency to breed in cold habitats. Its presence has only been detected from December to May, with the occasional company of *An. maculipennis* s.l. larvae. It has been documented that adults feed on birds (González et al., 2020) but do not appear to show interest in mammals, so they do not pose a threat to humans (Becker et al., 2020).

Culex pipiens, *Cx. theileri* and *Cs. annulata* are well distributed throughout Rías Baixas, on the west coast, showing preference for urban spaces as this is one of the areas with the highest population density of Galicia (Ramil et al., 2007) (Figure 3). The common mosquito *Cx. pipiens* is widely distributed in Spain (Bueno-Marí et al., 2012) and Galicia (Polina et al., 2022). Larvae breed preferably in artificial containers such as ditches, flower pots and buckets (Weitzel et al., 2014), so exclusively sampling in natural water bodies has greatly reduced its chance of capture in the present study. They were found at different altitudes, temperatures and pH values, being remarkable in their tolerance to high levels of conductivity and low levels of dissolved oxygen (even below 1 mg/L) (Table 2). They have been observed from April to September sharing larval habitat with *Cx. territans*, *Cx. theileri*, *An. maculipennis* s.l., *Cx. impudicus*, *Cs. annulata* and *Cq. buxtoni*. *Cx. pipiens* plays an important role as an arbovirus vector to humans in Europe, being a competent transmitter of WNV, Sindbis and Usutu Virus (USUV) (Becker et al., 2020).

Culex theileri is a species of Palearctic distribution well represented in Spain and with a known presence in the four Galician provinces (Bueno-Marí et al., 2012; Polina et al., 2022). Larvae have been collected in coastal lagoons, continental lagoons and temporary ponds with high levels of anthropization throughout the provinces of A Coruña, Lugo and Pontevedra (Figure 3, Table 1). Likewise, they have been found breeding in a wide altitudinal and physico-chemical variable ranges, tolerating alkaline and saline waters with high conductivity (Table 2). This is not surprising since this mosquito breeds in a wide variety of natural and artificial habitats, even in those that are strongly polluted (Becker et al., 2020; Ramos et al., 1978). *Cx. theileri* were collected in early summer, from June to July, sharing breeding sites with *An. maculipennis* s.l. and *Cx. pipiens* larvae. The females are zoophilic but sometimes feed on humans and can transmit some diseases such as Rift Valley fever virus and canine *Dirofilaria* (Becker et al., 2020; Ramos et al., 1978).

Culiseta annulata is a species of Palearctic and Afrotropical distribution (Edwards, 1921), common in Spain and Galicia (Bueno-Marí et al., 2012; Polina et al., 2022). However, in this study it has only been detected in coastal and continental lagoons of A Coruña and Pontevedra, in urban or peri-urban areas with high levels of anthropization (Figure 3) and always at low altitudes (Table 2). This species can breed in a wide variety of habitats, both natural or artificial, temporary or permanent and in open and shaded situations (Becker et al., 2020). Although it has been documented that the larvae can survive in extremely cold waters (López, 1989), they have not been collected below 15°C. They were observed from April to July sharing habitat with *An. maculipenis* s.l., *Cx. pipiens* and *Cq. buxtoni*. *Culi annulata* feed on humans, other mammals and birds (Becker et al., 2020), and it is a potential vector of Tahyna virus (Ribeiro et al., 1988) and avian malaria (Schaffner et al., 2001).

Other species recorded in smaller numbers are *Cx. territans*, *Cq. buxtoni* and *Cx. impudicus* (Table 2). *Cx. territans* is widely distributed throughout Europe (Becker et al., 2020) and Spain (Bueno-Marí et al., 2012), being also present in the four Galician provinces (Figure 3). Larvae preferably breed in permanent water bodies with dense vegetation and in cold waters in shaded situations (Mohrig, 1969), which coincides with this study (Figure 3, Table 2). Although larvae are rarely found in heavily polluted waters (Becker et al., 2020), exceptionally they have been recorded in Gándaras de Budiño, an area contaminated by industrial discharges with minimal levels of dissolved oxygen in water. Larvae were found from May to September sharing breeding sites with those of *An. maculipennis* s.l., *Cx. pipiens* and *Cx. impudicus*. They do not pose a risk to human health since they predominantly feed on amphibians, reptiles and birds (Becker et al., 2020).

Coquillettidia buxtoni is present in the Mediterranean subregion of the Palearctic, but its known distribution is limited to a few European countries, among which is Spain (Becker et al., 2020; Bueno-Marí et al., 2012). In Galicia, it has been detected in a single area per province (Figure 3) and at different altitudes (Table 2), both at high- and low-anthropization levels. It has been mainly recorded in continental lagoons such as Gándaras de Budiño, as well as in the coastal and highly brackish lagoon of Vixán. The biological data of this species are scanty due to its limited distribution range and rare findings (Becker et al., 2020), so the contributions made in this study may be of special interest. Larvae were collected in cold and neutral waters, being able to tolerate those slightly acidic and highly conductive (Table 2), as well as heavily polluted waters. *Coquillettidia* larvae and pupae obtain oxygen from the submerged parts of aquatic plants (Becker et al., 2020) and it should be considered that *Cq. buxtoni* could do this from *Typha* sp. (Typhaceae), *Phragmites* sp. (Poaceae) and *Juncus* sp. (Juncaceae) since the breeding sites where it was found were rich in these aquatic plants. It has been suggested that the widely contrasted dipping technique for mosquito sampling (Service, 1993) is not suitable for capturing *Coquillettidia* larvae due to its biology (Bueno-Marí, 2010). Given the positive results obtained in this study, we recommend vigorously stirring the substrate and the vegetation with an entomological net to detach and capture these mosquito larvae. They were found from February to September in company of *An. maculipennis* s.l., *Cs. annulata*, *Cx. pipiens* and *Cx. theileri* larvae. The females bite humans and cattle (Gutsevich et al., 1974), but there is no evidence that they can transmit disease-causing pathogens.

Culex impudicus is mainly distributed in the Mediterranean region, including Spain (Becker et al., 2020; Bueno-Marí et al., 2012) and three of the Galician provinces (Figure 3). Specimens have been found in two coastal lagoons and one continental lagoon at different altitudes, both in low- and high-anthropization areas. Although this species breed in fresh and well-shaded water bodies (Aitken, 1954), it has been observed in brackish waters in open situations. Likewise, larvae have been found in warm or slightly cold waters, well oxygenated and even in those with high levels of conductivity (Table 2). They occurred from July to September with *Cx. pipiens*, *Cx. territans* and *An. maculipennis* s.l. larvae. The females of *Cx. impudicus* feed on amphibians (Becker et al., 2020), and there is no evidence that they can play a role as vector of diseases.

Only one specimen was captured for *Ae. caspius* and *Cs. morsitans*, so their known distribution is restricted to a single place in the study area: the lagoon of Muro and the lagoon of Gándaras de Budiño, respectively (Figure 3). *Ae. caspius* is a Palearctic species (Becker et al., 2020) widely distributed in Spain but limited to the west coast in Galicia (Bueno-Marí et al., 2012; Polina et al., 2022). It is a halophilic mosquito, so it is not surprising that it was found in the brackish lagoon of Muro. Although the breeding site recorded in Portugal ranged from pH 6.0 to 7.0 (Pires et al., 1982), the specimen in the study area has been collected in basic waters (Table 2). The larvae were captured in April 2007 and in the absence of other mosquito species. The females readily bite humans and other animals (Gutsevich et al., 1974), being a potential vector of some diseases such as Tahyna virus (Becker et al., 2020).

Culiseta morsitans is a species of Palearctic distribution common in Europe (Becker et al., 2020), but with a reduced known distribution in Spain (Bueno-Marí et al., 2012; González et al., 2020; Ruiz-Arrondo et al., 2017). In Galicia it has only been detected in a continental lagoon of Gándaras de Budiño (Figure 3), characterized by marshy and highly polluted waters. The obtained results reinforce the fact that larvae preferably breed in swampy woodlands and tolerate slightly brackish and cold waters (Table 2). The specimen was captured in December 2003 without the company of other mosquito larvae. Females mainly feed on birds and occasionally on reptiles and small mammals (Becker et al., 2020), being a potential vector of Ockelbo virus (Francy et al., 1989).

The result of the calculated metrics (N, S, H′ and DS) pooled by groups reveals that the greatest differences were found within water body types, while differences were not clear among anthropization levels and the two climatic zones (Table 3, Table 4). Contrary to what happens with abundance, the species richness of mosquitoes is higher in lagoons than in temporary ponds. The values of Shannon–Wiener Diversity Index are low in all cases ($H' < 2$), indicating a poor heterogeneity of mosquitoes in the study area. The Simpson Index varies slightly between groups, showing the highest dominance values in coastal lagoons and in highly anthropized areas (DS >0.5). It is precisely in these sites where there is a larger proportion of *Cx. pipiens* and *Cx. theileri* (Figure 3), which are well adapted to breed in a great diversity of breeding sites (Becker et al., 2020).

Bueno-Marí et al. (2014) have concluded that certain human influence may promote mosquito diversity. Although the present study shows a major species richness in highly anthropized areas (Table 3), concentrated on the west coast and the southern border of the region (Figure 3), it is not possible to reach any clear conclusion since no statistically significant differences have been detected (Table 4). The fact that only natural habitats have been studied is decisive for drawing conclusions, since the diversity of culicids is related to the availability of artificial breeding sites (Bueno-Marí et al., 2014).

The study of mosquitoes at local and regional levels is essential to adopt the most responsible and efficient measures of prevention and control against possible health risks. The present study not only contributes to the knowledge of the distribution and diversity of mosquitoes in Spain, but also expands the information available on their ecology and breeding habits, especially in the case of little studied species such as *Cq. buxtoni*. Likewise, this study highlights the existence in northwestern Spain of anthropophilic species capable of acting as vectors of pathogens such as *An. maculipennis* s.l., *Cx. pipiens*, *Cx. theileri* and *Ae. caspius*. Whereas the ubiquity of *Cx. pipiens* is already known, what is surprising is the wide distribution and frequency of *An. maculipennis* s.l. in the study area. Although their predominance in rural environments reduces the number of human–mosquito encounters and health risk associated with each bite, the monitoring of their populations should not be neglected. In fact, it would be of particular interest to develop future studies that incorporate molecular identification to reveal which species of the *An. maculipennis* s.l. complex are present in the region and, therefore, clarify the health risk of malaria transmission.

The fact that the data provided by this study is the result of different water monitoring projects reinforces the benefits of collaborative work on environmental health issues. In this way, it is possible to take advantage of resources and efforts with a common objective, as well as to increase knowledge of those mosquito species which are difficult to capture by conventional sampling methods.

AUTHOR CONTRIBUTIONS

Yasmina Martínez-Barciela: Investigation; writing – original draft; writing – review and editing; data curation; formal analysis; methodology; resources; funding acquisition; conceptualization. **Alejandro Polina:** Investigation; writing – review and editing; resources; methodology. **Josefina Garrido:** Supervision; project administration; methodology; validation; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interests to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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