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Population Dynamics and Seasonal Patterns of *Chironomus plumosus* (Diptera, Chironomidae) in the Shallow Lake Trasimeno, Central Italy

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Abstract: Field sampling of littoral macrobenthos of the shallow Lake Trasimeno was conducted along 17 years (2005–2021) on 129 different occasions. This long-term field study deepens the knowledge concerning the life cycle of *Chironomus plumosus* (Diptera), the main responsible for summer chironomid swarms that adversely affect human littoral activities, providing useful information for its management. About 108,000 macrobenthic specimens were collected, belonging to Oligochaeta (Naididae) (62%), Diptera (Chironomidae) (37%), and only 1.5% to other invertebrate taxa. Eighteen chironomid taxa were found. The trend of chironomid density was not affected by *C. plumosus*, which showed a maximum increase in September. This peak is justified by the presence of large swarms of *C. plumosus* in late August in which the populations of the central area of Lake Trasimeno consistently participate. The larval density of this species did not increase over the 17 years. A detailed analysis of the sampled larvae and adult biomass catches from 2017 to 2020 reveals that four annual swarmings occurred: in April, July, August, and September–October. The water temperature remains higher than 20 °C during the night hours from the end of May to mid-September, strengthening the hypothesis of the three midge swarming cycles in the summer period until early autumn.

Keywords: chironomids; larval instar; midge swarms; macrobenthos communities; lake's littoral zone; temperature

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1. Introduction

Freshwater ecosystems are an essential component of the ecosphere, significantly contributing to the maintenance of its biodiversity despite constituting less than 1% of the Earth's surface; the 126,000 known freshwater animal species represent approximately 10% of the described animal species [1,2]. These ecosystems are at risk of changing rapidly; the major drivers of these changes are climate modifications, land-use, and cover alterations, chemical inputs, the introduction of invasive aquatic species, and other human exploitation practices, such as aquaculture [3].

Benthos of lakes significantly contributes to total lake productivity and biodiversity, especially in shallow lakes [4–7]. Macroinvertebrates, inhabiting the surficial layer of the bottom sediments, are tightly integrated into the structure and functioning of the benthic habitat [8,9], where they constitute an essential fraction of secondary production and are a critical component of the fish diet [10]. Benthic macroinvertebrates can also serve as valid biological indicators of environmental quality because they are subject to the resulting action of pollution and thus respond to many ecological stressors [11,12].

Chironomids (Insecta, Diptera) are the aquatic insect family with the highest number of species [13]. They constitute a relevant component of the benthic biocoenosis of freshwater ecosystems, being usually dominant, particularly in lakes [14–16]. They are also known as "non-biting midges" and are typical holometabolous insects with four larval stages (instars). Chironomids are the subject of research in many fields, such as genetics, biochemistry, biodiversity, phylogenetics, and biogeography [17]; they are also frequently used as test organisms for ecological risk assessment, and the evaluation of mouthpart deformities of their larvae is fairly a widespread tool for sediment toxicity assessment [18–20]. Chironomids, unlike other Culicomorpha, do not raise concerns in the human health field because they are not hematophagous. However, their massive swarms do have an adverse impact on human activities, such as the tourism industry (economic losses), and also cause soiling of surfaces and monuments, hazards in vehicular and air traffic, and to a lesser extent, allergies or conjunctivitis [21–23]. Many chironomid species are documented as pestiferous; Ali (1991) [24] mentioned that about 100 out of the nearly 4000 known chironomid species are pestiferous, with the most recurrent species posing nuisance and economic problems belonging to the genus *Chironomus*, in particular *Chironomus plumosus* (Linnaeus, 1758), one of the world's most ubiquitous species in eutrophic lakes, ponds and reservoirs [15,23,25]. Occurrence of pestiferous chironomid events has been registered all over the world, both in natural water bodies and man-made aquatic infrastructures, in Europe (Cyprus, England, Germany, Italy, and Switzerland), Asia (Israel, Japan, Singapore), Africa (Ghana and Sudan), North America (USA: California, Florida, Georgia, and Ohio), and South America (Brazil) [15,23].

Similarly, since the early 2000s, Lake Trasimeno (Umbria, central Italy) has witnessed intense summer chironomid swarms resulting from an increase in summer temperatures and a decrease in rainfall, which favored water eutrophication [26]. Waterfront residents, tourists, accommodation facilities, businesses, and other commercial activities are adversely affected by this annoyance [27]. For these reasons, since 2004, there have been several chironomid control and containment activities funded by local administrations and private companies aimed at reducing the nuisance created by these swarms. These diversified actions were taken in trying not to damage the lacustrine ecosystem. The main activity carried out to control the larval populations of these insects concerned the use of biological larvicide, *Bacillus thuringiensis* var. *israelensis* (*Bti*), limited only to the summer period and to the littoral area of Lake Trasimeno. The control activities against adults varied over the years. They included, at first, the use of low-toxicity insecticides as adulticides, and then various adult behavioral and more ecologically oriented activities. The latter consisted of (i) diversion of adult midges from inhabited areas using outdoor lighting (Tofo-Lamps) set up along the shoreline [28], (ii) use of bats as biological control, through the waterfront installation of numerous bat boxes, and (iii) installation of midge traps, based on light attraction. These traps can collect huge amounts of adult chironomids through a suction system.

The present work has been aimed to investigate Lake Trasimeno's littoral macrobenthic community, focusing on the chironomid populations, with particular attention on exploring the *Chironomus plumosus* life cycle. This long-term study (2005–2021) is the first investigation on the chironomid littoral benthic community at Lake Trasimeno that deepens the knowledge of *C. plumosus* population dynamics and seasonal patterns, as the main midge species responsible for summer swarms that adversely affect human littoral activities. This study will provide useful information for the population management of this pestiferous species.

2. Materials and Methods

2.1. Study Area

Lake Trasimeno (geo-coordinates: 43°08′ N, 12°06′ E), is a shallow lake of tectonic origin located in the north-western portion of Umbria, central Italy, at 257 m above sea level (a.s.l.) (Figure 1). Recent studies suggest that the lake originated first as a marine gulf in the continental shelf of the Tyrrhenian Sea, then evolved into a wide fluvial plain, and finally became a freshwater lake filling a subsiding tectonic depression (Middle Pleistocene to present) [29]. This lake is the largest lake in the Italian Peninsula and the fourth largest in the whole country. The lake has an extensive surface area covering 124 km^2, a perimeter of 52 km, and a relatively limited catchment area covering 269 km^2. The average and maximum depths of the lake are 4.7 and 6.3 m, respectively [30].

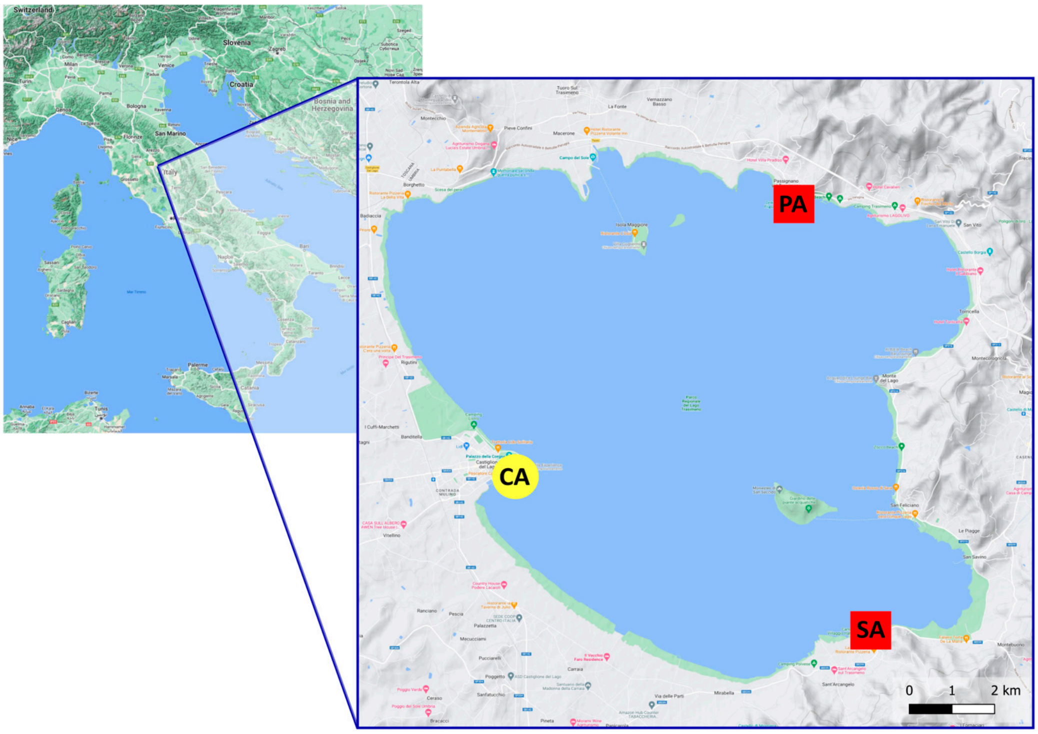

Figure 1. Lake Trasimeno: study area and sampling sites (CA, Castiglione del Lago; PA, Passignano sul Trasimeno; SA, S. Arcangelo).

Lake Trasimeno is a closed basin lake, having no natural outlets, and its hydrological regime depends on local precipitation, showing considerable fluctuations in the water level over the years [31,32].

The recurring flooding problem of Lake Trasimeno was definitively resolved in 1898 through the construction of the new S. Savino emissary that conveys the waters above the "hydrometric zero" (257.33 m a.s.l.) into the Tiber River basin. The drastic decrease in the water level of the lake, which in the 1950s reached 254.70 m a.s.l. (2.63 m below the threshold with a maximum water depth of 2.98 m), is a complex problem to resolve. During this period, an increase in the number of shallow swamps was conducive to a massive invasion of hydrophytes accompanied by nocturnal anoxia and daytime oxygen oversaturation. During 1959–1961, the realization of the artificial inlet, Anguillara channel, conveying the Tresa, Rio Maggiore, Moiano, and Maranzano streams into the lake, resulted in an increase in the catchment area of about 75 km^2 [31]. This intervention, conducted during the 1960s, allowed for regular water level maintenance until the end of the 1980s. However, it has been observed that since the early 1990s, the water level in the Trasimeno has been continuously declining and it is currently reaching about 256.00 m a.s.l. (Figure 1). At times when the emissary is not functioning properly, the lake water level decreases due to evaporation, and as a result, the dissolved salts increase in the water [33].

The area is characterized by a Mediterranean climate, with maximum rainfall in autumn and a minimum in summer. The lake is basically mesotrophic, and it is characterized by a flat and silty-sandy bottom [26]. The littoral zone of the lake is colonized by various hydrophytic, marsh, and transition plant associations; the reed bed is widely represented in the less anthropized stretches [34].

Lake Trasimeno is an important biotope, primarily from a historical and naturalistic point of view and has been identified as Special Area for Conservation (IT5210018; Habitat Directive, 1992/43/EEC), Special Protection Area (IT5210070; Bird Directive, 2009/147/EC), and Regional Natural Park.

The macroinvertebrate community of Lake Trasimeno is characterized by a diverse assemblage of benthic taxa, such as annelids, molluscs, crustaceans, and insects, rich in non-indigenous species (e.g., *Procambarus clarkii*, *Dikerogammarus villosus*, *Dreissena polymorpha*, *Sinanodonta woodiana*) [20,35–37].

The littoral area of Lake Trasimeno, in particular in correspondence with towns, accommodation facilities, and other commercial activities (approximately up to 30 m from the coast, total area of about 400 ha), has been treated during the summer (at least 5 treatments per year, almost every year, since 2005), with an average of 5000 L per year of a biological larvicide in aqueous suspension, based on the active ingredient *Bacillus thuringiensis* var. *israelensis* (*Bti*), through the use of an amphibious hovercraft by USL Umbria 1, as part of various control projects.

2.2. Sampling Campaign (Littoral macrobenthos)

Field sampling was conducted in the littoral zone of Lake Trasimeno, close to the town of Castiglione del Lago (CA), along a 350 m long stretch of the lake parallel to the coastline (1–1.5 m mean water depth) (Figure 1). In this area, six sampling sites, representative of the main bottom types (sandy, silty, and macrophyte-covered) of the littoral zone of the lake, were selected. During each sampling occasion, a mean of 5–6 quantitative sub-samples of the top 6–7 cm of the lake bottom sediments was collected from each sampling area (mean of 34 sub-samples per sampling occasion) with a hand dredge for shallow lakes with fine grain size bottoms (14 × 8 cm size, 112 cm^2 sampling surface).

The total number of surveys carried out was 129, spread over a period of 17 years, from 2005 to 2021 (Table 1). The field sampling was carried out with a variable frequency over the years. To be precise, a minimum of 1 sampling in 2005 and up to a maximum of 23 samplings during 2018 were made. An intensification of the field survey was made during the 2017–2020 period. Generally, field sampling was carried out more frequently during the summer months, with July being the most sampled (22 surveys), followed by June (19) and September (18); March was the least sampled month.

Table 1. Monthly surveys (2005–2021) of littoral benthos at Lake Trasimeno (CA, Castiglione del Lago); the intensification of the field survey in the years 2017–2020 is indicated in grey.

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total per Month
January	—	1	—	1	—	—	—	—	—	—	—	—	—	—	1	1	1	**5**
February	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	**3**
March	—	1	1	—	—	—	—	—	—	—	—	—	—	—	1	—	1	**4**
April	—	1	1	—	—	—	—	—	—	—	—	—	—	2	1	—	—	**5**
May	—	1	1	1	1	—	1	—	—	—	—	—	1	3	2	—	—	**11**
June	—	1	1	1	1	1	1	1	1	1	—	1	—	4	3	2	—	**19**
July	—	1	1	1	1	1	—	1	1	1	2	1	4	3	2	2	—	**22**
August	—	1	—	—	—	—	1	2	2	—	—	—	4	3	2	2	—	**17**
September	—	1	1	—	—	1	1	1	1	—	1	—	4	3	2	2	—	**18**
October	1	1	1	—	1	—	1	—	—	—	—	—	3	3	2	1	—	**14**
November	—	—	1	—	—	—	—	—	—	—	1	—	1	1	1	1	—	**6**
December	—	1	1	—	—	—	—	—	—	—	—	—	—	1	1	1	—	**5**
Total per year	**1**	**10**	**9**	**4**	**4**	**3**	**5**	**5**	**5**	**2**	**4**	**2**	**17**	**23**	**19**	**13**	**3**	**129**

Field-collected bottom sediments were taken to the laboratory in plastic containers. In the laboratory, each sample was sieved (washed), and benthic macroinvertebrates were separated, sorted, and preserved in 70% ethanol. The benthic community of each sample was identified at the family level using suitable taxonomic keys [38]. Specimens belonging to the Chironomidae family were identified to the genus level, except for *Chironomus plumosus*, identified to the species level. The identification was made through the observation, on microscope slides, of peculiar features of the chironomid head capsules with suitable taxonomic keys [17,39–42]. For each chironomid specimen, body length (head, thorax, and abdomen) and cephalic capsule length and width were measured to determine the larval development stage (instar).

2.3. Sampling Campaign (Adult chironomids)

To understand and analyze the adult swarming cycles of chironomids, the adults were collected by using an experimental device named ChiroTrap (registered trademark). This trap has been developed by the Italian Company, Laika Lab s.r.l., as part of a research project funded by the Brunello and Federica Cucinelli Foundation ("Chironomid populations control at Lake Trasimeno: evaluation and development of biological control methods and new systems for mechanical-light attraction and capture"), Italy.

The ChiroTrap is designed to attract and trap adult insects, such as adult Chironomidae. The trap is provided with a light system that exploits the positive phototactic behavior of some adult insects. The trap is equipped with a suction chamber with an opening of 1×1 m. Two ChiroTraps were employed along the coast of Lake Trasimeno, one located at Sant'Arcangelo (SA) and the other at Passignano sul Trasimeno (PA) (Figure 1) and were activated during the spring to early autumn periods of 2018, 2019, and 2020, respectively, for 135 (SA) and 80 (PA), 273 (SA) and 360 (PA), and 203 (SA) and 287 (PA) days. The ChiroTrap is equipped with sensors that continuously (every minute) detect the weight of the captured biomass (predominantly adult chironomids and some aquatic insects), air temperature, water temperature, relative humidity, and wind direction and intensity.

Of the data collected from 2018 to 2020 by using the field trap, air and water temperatures and the weight of the collected biomass recorded around sunset (from 6 p.m. to 11 p.m.; [43]) were considered for analysis.

3. Results

During the 17 years of field sampling from 2005 to 2021 concerning the littoral macrobenthos of Lake Trasimeno, field collections were made on 129 occasions in the sample area of Castiglione del Lago (Table 1). A total of about 108,000 specimens belonging to Oligochaeta (Naididae) (61.99%), Diptera (Chironomidae) (about 36.54%), and only 1.48% belonging to other faunal components, such as Bivalvia, Crustacea, and other Insecta were collected (see Supplementary Material). The average monthly density of all macrobenthos amounted to about 1984.54 individuals m^{-2} (ind. m^{-2}), with Naididae and Chironomidae, respectively, amounting to 1242.55 and 710.24 ind. m^{-2}. All other faunal components amounted to 31.74 ind. m^{-2} (Table 2).

The analysis of the littoral chironomid populations revealed the presence of 18 taxa. The taxa with the highest average monthly density and percentage distribution were *Polypedilum* sp. with 191.65 ind. m^{-2} and 36.53% (95% recurrence), *Cladotanytarsus* sp. with 93.07 ind. m^{-2} and 17.74% (recurrence of 71%), *Chironomus plumosus* with 85.95 ind. m^{-2} and 16.38% (recurrence of 96%), and *Stictochironomus* sp. with 65.64 ind. m^{-2} and 12.51% (83% recurrence) (Table 3), respectively. In addition, these four taxa occurred during all 12 months of the year, together with *Cryptochironomus* sp. and *Dicrotendipes* sp. (Tables S1 and S2).

Table 2. Monthly mean density, standard deviation, and relative percentage (2005–2021) of Chironomidae, Naididae, and other taxa inhabiting Lake Trasimeno littoral zone (CA, Castiglione del Lago).

	Samplings	Chironomidae		Chironomidae	Naididae		Other Taxa		Total Macroinvertebrates
	(N)	(ind. m^{-2})	(%)	Taxa (N)	(ind. m^{-2})	(%)	(ind. m^{-2})	(%)	(ind. m^{-2})
January	5	381.00 ± 307.87	28.54	11	942.60 ± 816.33	70.60	11.60 ± 19.22	0.87	1335.20 ± 1035.39
February	3	413.67 ± 252.69	27.38	10	1086.33 ± 538.54	71.91	10.67 ± 18.48	0.71	1510.67 ± 493.31
March	4	1138.00 ± 1218.91	64.58	11	603.75 ± 198.92	34.26	20.50 ± 31.42	1.16	1762.25 ± 1133.87
April	5	813.50 ± 597.13	52.39	9	736.88 ± 579.69	47.46	2.38 ± 4.75	0.15	1552.75 ± 140.54
May	11	793.33 ± 580.12	43.80	15	1007.04 ± 552.35	55.60	10.73 ± 14.27	0.59	1811.10 ± 914.38
June	19	1077.37 ± 736.09	53.90	15	892.04 ± 544.98	44.63	29.31 ± 37.54	1.47	1998.72 ± 975.68
July	22	904.38 ± 559.56	32.75	15	1792.25 ± 1659.22	64.90	65.01 ± 78.69	2.35	2761.64 ± 1919.53
August	17	600.05 ± 391.24	22.94	17	1947.21 ± 1438.64	74.46	67.97 ± 74.93	2.60	2615.23 ± 1659.55
September	18	541.03 ± 460.00	26.67	16	1453.91 ± 613.61	71.68	33.52 ± 30.48	1.65	2028.46 ± 938.54
October	14	620.43 ± 459.12	25.57	14	1767.74 ± 1551.17	72.87	37.78 ± 37.15	1.56	2425.94 ± 2001.96
November	6	405.33 ± 181.23	24.80	11	1189.67 ± 826.37	72.78	39.67 ± 40.92	2.43	1634.67 ± 914.17
December	5	834.80 ± 536.52	35.11	11	1491.20 ± 1075.22	62.71	51.80 ± 71.88	2.18	2377.80 ± 1444.59
mean	**10.75**	**710.24 ± 257.03**	**36.54**	**12.92**	**1242.55 ± 441.10**	**61.99**	**31.75 ± 21.76**	**1.48**	**1984.54 ± 465.91**
min	3	381.00	22.94	9	603.75	34.26	2.38	0.15	1335.20
max	22	1138.00	64.58	17	1947.21	74.46	67.97	2.60	2761.64

Table 3. Mean density, standard deviation, and recurrence (2005–2021) of Chironomidae taxa of the Lake Trasimeno littoral zone.

	Samplings	Recurrence	Density	
Taxa	(N)	(%)	(ind. m^{-2})	(%)
***Polypedilum* sp.**	122	94.57	191.65 ± 107.95	36.53
***Cladotanytarsus* sp.**	91	70.54	93.07 ± 122.05	17.74
Chironomus plumosus	124	96.12	85.95 ± 75.37	16.38
***Stictochironomus* sp.**	107	82.95	65.64 ± 58.13	12.51
***Cryptochironomus* sp.**	124	96.12	32.68 ± 15.19	6.23
***Tanypus* sp.**	74	57.36	13.12 ± 18.37	2.50
***Cladopelma* sp.**	64	49.61	9.70 ± 12.45	1.85
***Dicrotendipes* sp.**	84	65.12	8.65 ± 4.48	1.65
***Tanytarsus* sp.**	54	41.86	8.0 ± 12.08	1.52
***Microchironomus* sp.**	59	45.74	5.34 ± 8.33	1.02
***Procladius* sp.**	60	46.51	4.59 ± 7.67	0.88
***Glyptotendipes* sp.**	43	33.33	4.16 ± 3.49	0.79
***Eukiefferella* sp.**	18	13.95	1.53 ± 2.03	0.29
***Pseudochironomus* sp.**	10	7.75	0.24 ± 0.34	0.05
***Microtendipes* sp.**	7	5.43	0.14 ± 0.25	0.03
***Endochironomus* sp.**	4	3.10	0.14 ± 0.28	0.03
***Cryptotendipes* sp.**	6	4.65	0.07 ± 0.15	0.01
***Parachironomus* sp.**	2	1.55	0.02 ± 0.05	0.00

The average monthly density of Chironomidae and *C. plumosus* is shown in Figure 2. The trend of chironomids was not affected (r = −0.21) by the density of the *C. plumosus* population, which, unlike the whole chironomid community, showed a maximum increase with average values of 175.76 ind. m^{-2} during summer and early autumn (July, August, September, and October), with a peak of 249.44 ind. m^{-2} in September, during which *C. plumosus* represented the 46.10% of the total Chironomidae and the 12.30% of the entire macrobenthic community.

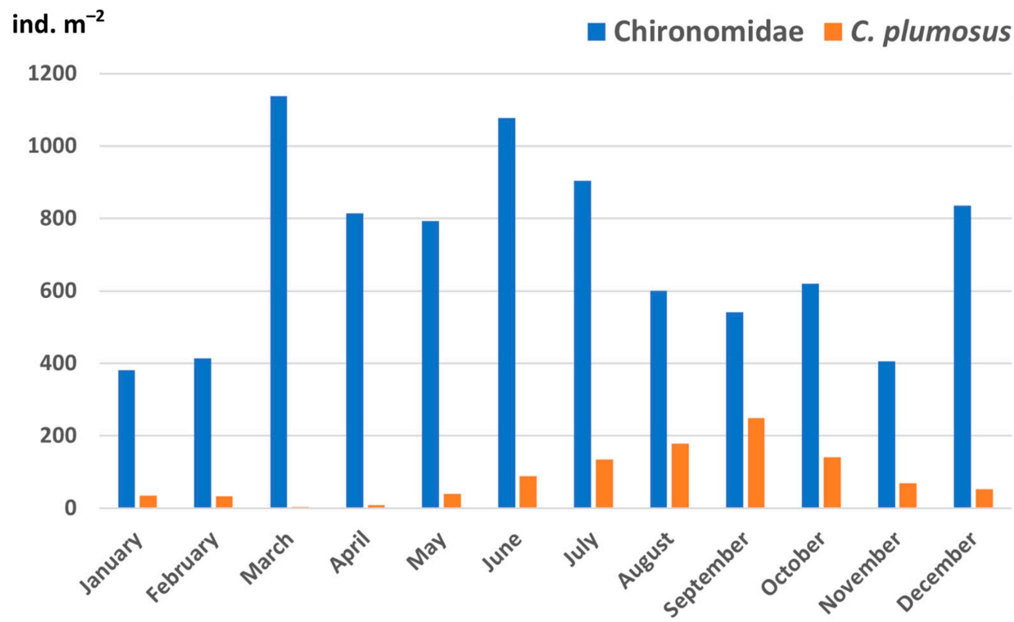

Figure 2. Chironomidae (Diptera) and *Chironomus plumosus* monthly mean larval density (2005–2021) in the Lake Trasimeno littoral zone.

A comparison of the mean monthly larval density of *C. plumosus* over the 17 years (Figure 3) of the study showed that during the summer season, this species had the greatest population expansion, but the larval density of this chironomid species did not appear to be increasing over the years, although reaching high values similar to the initial years of the survey only in 2019.

The results of this field survey (2005–2021) revealed that three chironomid taxa (previous research [28]) responsible for the insect swarms around artificial lights, *C. plumosus*, *Tanypus punctipennis* and *Procladius* sp. (probably *P. choreus*), amounted altogether to a mean of about 35.38% (241.25 ind. m^{-2}) of the chironomid larvae during the summer period (July, August, and September). Their relative population percentages were 78%, 16%, and 6%, respectively.

Table 4 indicates that *C. plumosus* larvae overwinter in the mature stage during January, February, and March. During these months only 4th instar larvae, with the rare presence of 3rd instars, occurred. The first collection of 2nd instar larvae of this species during the year occurs in April. The first appearance of the swarms during the year is in spring (April).

Table 4. *Chironomus. plumosus* larval instars (2, second; 3, third; 4, fourth or mature larval stage) in the different months at the Lake Trasimeno littoral zone (2005–2021); the intensification of the field survey in the years 2017–2020 is indicated in grey.

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
January	-	-	-	3 4	-	-	-	-	-	-	-	-	-	-	4	4	(4)
February	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(3) 4	4	(3)
March	-	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
April	-	-	(2) 4	-	-	-	-	-	-	-	-	-	-	2 (3)	2 4	-	-
May	-	3	2 3 4	(2) 4	3 4	-	2 3 4	-	-	-	-	-	2 3 4	2 3 4	2 3 4	-	-
June	-	2 (3) (4)	2 3 4	2 3 4	3 4	2	2 3 4	2 3 4	(3) (4)	2 3 (4)	-	2 3 4	-	2 (3) 4	2 3 4	2 3 4	-
July	-	2 3 4	2 3 4	2 3 4	2 3 4	2 3 4	-	(2) 3 4	2 3 4	2 3 4	2 3 4	3 4	2 3 4	2 3 4	(2) 3 4	(2) 3 4	-
August	-	2 3 4	-	-	-	-	2 3 4	3 4	2 3 4	-	-	-	2 3 4	2 3 4	2 3 4	2 3 4	-
September	-	(2) 3 4	3 4	-	-	2 3 4	(2) 3 4	2 3 4	2 3 4	-	2 3 4	-	(2) 3 4	2 3 4	2 3 4	2 3 4	-
October	2 3	2 3 4	3 4	-	3 4	-	2 3 4	-	-	-	-	-	(2) 3 4	2 3 4	2 3 4	3 4	-
November	-	-	2 (3) 4	-	-	-	-	-	-	-	(2) 3 4	-	(3) 4	4	3 4	4	-
December	-	2 3 4	4	-	-	-	-	-	-	-	-	-	-	3 4	(3) 4	(3) 4	-

The presence of only one specimen is indicated by parentheses

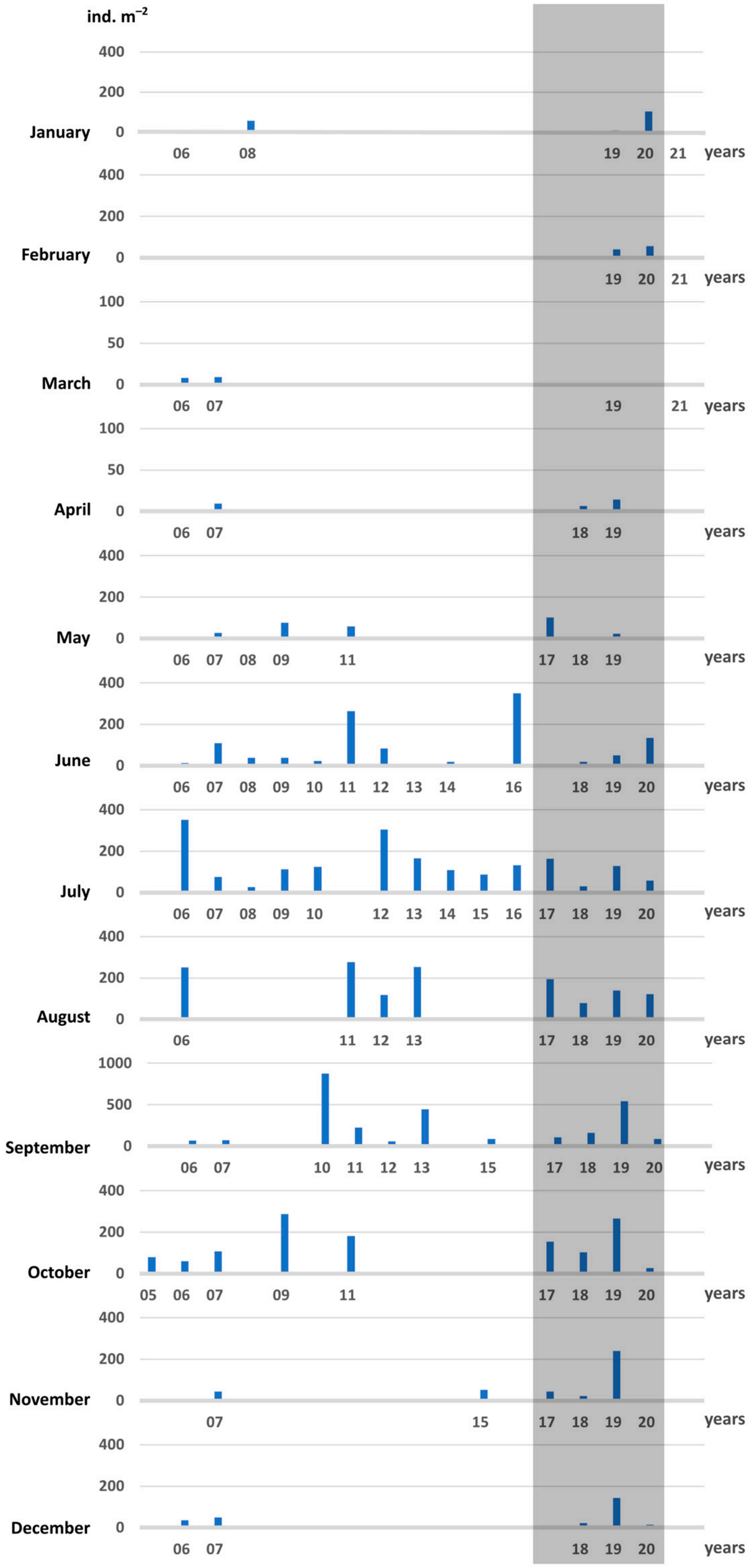

Figure 3. Monthly mean larval density of *Chironomus plumosus* (2005–2021) in the Lake Trasimeno littoral zone. Only the years subject to monitoring are indicated (example: 05 corresponds to 2005); the intensification of the field survey in the years 2017–2020 is indicated in grey.

From the examination of the weekly data (from May to October) derived from the surveys carried out during the years 2017–2020 (Figure S1), it appears that several *C. plumosus* life cycles are completed during the year. The abundance of 2nd instar larvae in early July, in mid-late August, and in late September–mid-October, implies new egg depositions connected to relative swarmings (Table 4).

To validate these results, adult chironomid collections made continuously by ChiroTraps from April to October of the years 2018–2020 were also examined. It was discovered that light attraction affected chironomids almost exclusively. In the PA ChiroTrap, the biomass of fauna other than chironomid taxa amounted only to 0.35%, while in the SA ChiroTrap it amounted to 3.41%. It was also verified and confirmed that among the chironomids, *C. plumosus* predominated in the trap collections. Figure 4 shows the biomass captured on a weekly basis between 6 p.m. and 11 p.m. at PA in 2020, which had the most efficiency as site and year for data collection and was less influenced by other fauna. As shown in Figure 4, the four annual swarmings occurred during the same seasonal periods highlighted in Figure S1.

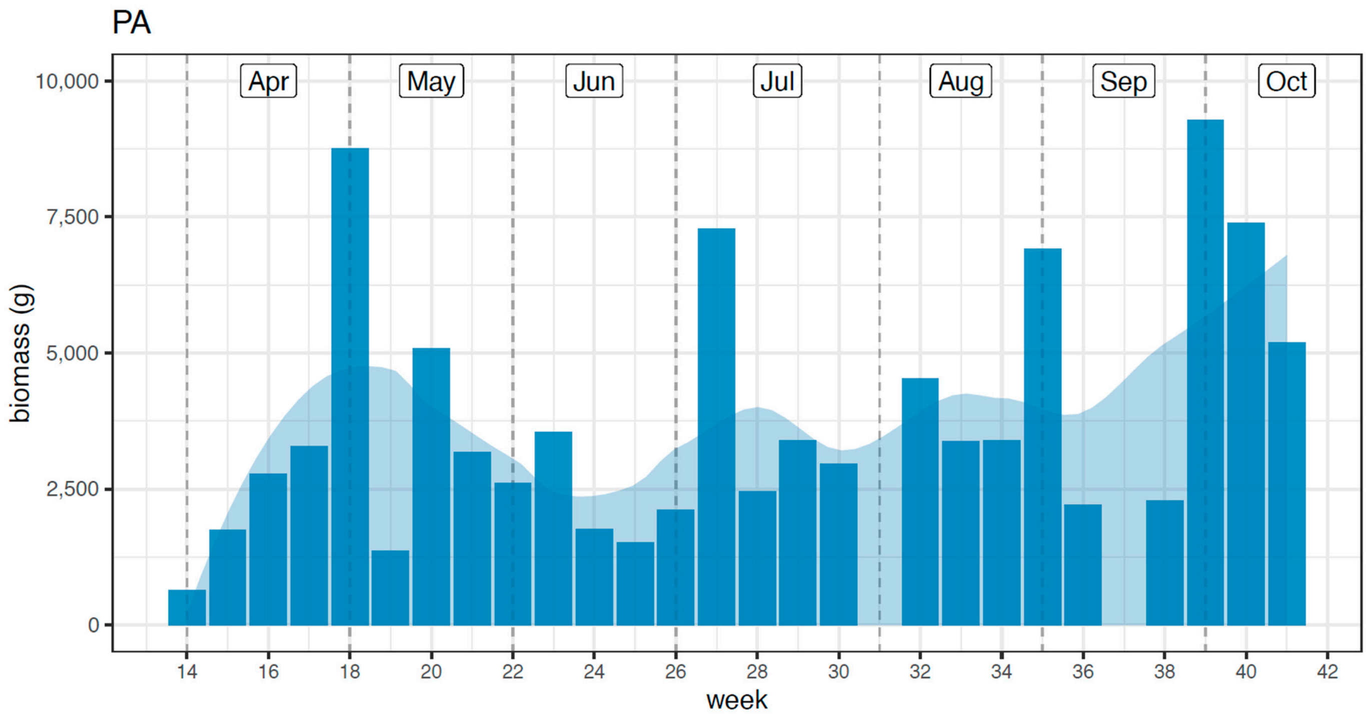

Figure 4. Adult chironomid biomasses collected with the ChiroTraps at Passignano sul Trasimeno (PA) site from the 14th to the 41st week of the year 2020.

Insect cycles, like those of all ectotherm animals, are closely related to environmental temperature. In this regard, the instrumentation installed in the ChiroTrap allowed to continuously monitor air and water temperature. Figure 5 shows the trends of average, minimum, and maximum weekly temperatures, for the year 2019, to align them with the concomitant collections of chironomid biomass attracted by the lights. Overall, for the year 2019, which presents the most homogeneous data collection both in Passignano sul Trasimeno (PA) and in S. Arcangelo (SA), the mean values for air temperature were 17.18 °C with an average variation of 19.73 °C, while those for water temperature were 16.50 °C, with an average variation of 4.22 °C.

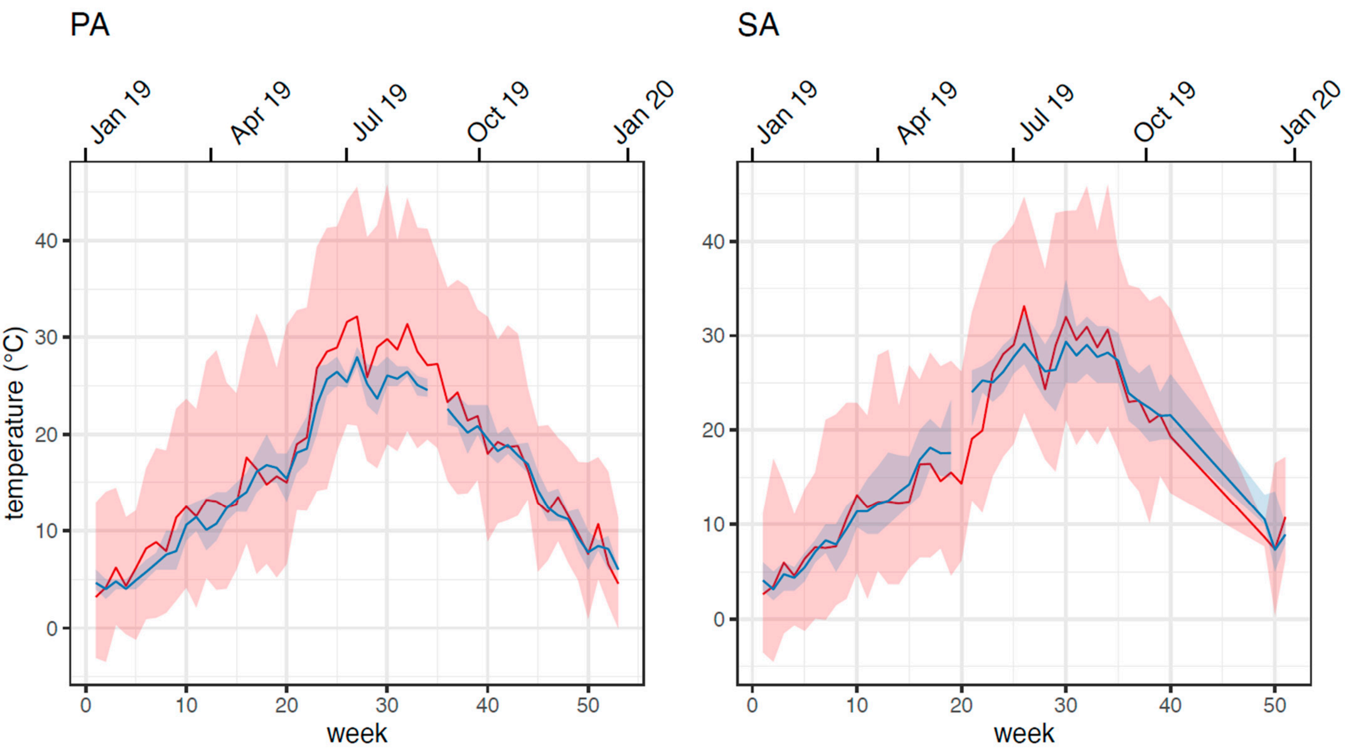

Figure 5. Air and water temperature weekly mean (2019) measured in the littoral zone of Lake Trasimeno with the ChiroTraps at Passignano sul Trasimeno (PA) and S. Arcangelo (SA). Red lines indicate the mean air temperature (min and max oscillation in pink), blue lines indicate the mean water temperature (min and max oscillation in grey).

4. Discussion

The study, during the 17 years of investigation (2005–2021: 129 field collection occasions), allowed to evaluate the macrobenthic littoral biocenosis of Lake Trasimeno in the sample area of Castiglione del Lago (average monthly density of 1985 ind. m^{-2}) showing a low biodiversity of the macroinvertebrate community, constituted essentially of Oligochaeta (Naididae) (about 65%) and Diptera (Chironomidae) (about 34%). In the last decades, an evident transformation of the natural coast of the lake was observed in some littoral areas, including the sample area. The creation of recreational beaches along the coasts has determined an adverse effect on the littoral biodiversity [44]. In fact, the reed vegetation that was initially characterizing the area has suffered a reduction of over 65% in the whole lake between 1988 and 2005. However, this kind of distribution of the benthic macrofauna, consisting mainly of Oligochaeta and larvae of Chironomidae, is typically found in small shallow lakes, such as Shoji Lake, the smallest of the Fuji Five Lakes (Japan) [45], as in large shallow lakes, such as Lake Balaton [46].

In the summer period (2005–2021), most of the littoral zone of Lake Trasimeno (with a water depth of less than 1.5 m) was subjected to the control of the chironomid populations by the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*), a specific biological larvicide for dipterans [23,27]. From the data, it is not possible to infer how much the use of *Bti* during these 17 years has reduced and contained the larval populations of chironomids in the littoral area, especially of *C. plumosus*, the most problematic species. However, during the summer season of the whole 17 years examined, this species population does not show any increasing trend (Figure 3). Despite the *Bti* treatments, among chironomids, a fair biodiversity is observed (18 taxa), with a monthly average of 13 taxa over the years.

Surprisingly, the share of the highest average monthly density (85.95 ind. m^{-2}) among the chironomid populations in the littoral area of Lake Trasimeno is not up to *C. plumosus*, instead it is up to *Polypedilum* sp., which has a much higher average density (191.65 ind. m^{-2}). In addition, *Cladotanytarsus* sp. shows a monthly average density

(93.07 ind. m^{-2}) greater than *C. plumosus*. However, these chironomid taxa are common, and sometimes dominant, in the littoral areas of lakes, and their observed densities are comparable to those from other shallow lakes [47].

On the other hand, *C. plumosus*, in the late summer period, from August to October, represents the chironomid taxon with higher density, particularly in September, when the density of its larvae is 4.5 times greater than that of *Polypedilum* sp. and about 11 times compared to *Cladotanytarsus* sp.

However, *Polypedilum* sp. and *Cladotanytarsus* sp. are not among the taxa attracted by artificial lights along the littoral area of Lake Trasimeno [28]. In this previous study, about 41,000 chironomid adult specimens attracted by various light sources (Tofo-Lamps, Province of Perugia and USLUmbria1, Italy) located in the littoral areas of Lake Trasimeno were monitored during the summer period (July, August, and September from 2007 to 2009). Goretti et al. (2011) [28] found that the taxa constituting the swarms attracted by the white artificial lights were essentially represented by *Chironomus plumosus* (67%), *T. punctipennis* (22%) and *Procladius* sp. (probably *P. choreus*, 9%) [48].

Taking into account only these three chironomid taxa, the results of the actual field survey (2005–2021) reveal that, during the summer period (July, August, and September), the three taxa show a distribution of the larval populations comparable to that of the populations constituting the swarms attracted by the artificial lights along the lake shoreline. In particular, *C. plumosus*: larvae 78% (2005–2021), vs. adults 67% (2007–2009); *T. punctipennis* larvae 16% (2005–2021), vs. adults 22% (2007–2009); and *Procladius* sp. larvae 6% (2005–2021), vs. adults 9% (2007–2009). These similarities in their allocations confirm that these three chironomid taxa are the main ones responsible for the swarms attracted by the artificial lights along the shores of Lake Trasimeno and, consequently, for the nuisance generated to the tourist and recreational activities.

Another unexpected fact is the negative correlation between the larval populations of total chironomids and that of *C. plumosus* during the warm season. In particular, September shows a regression of the larval populations of the other taxa of chironomids that show biological cycles with high larval density, especially in spring (from March to May).

The *C. plumosus* peak of larval density during September is justified by the great swarms of this species in late August, during which its populations living in the extended pelagic and central zone of Lake Trasimeno participate decisively. In fact, the chironomid populations from the central zone of Lake Trasimeno show a very high *C. plumosus* density, reaching 97% share of all chironomids (average density 335 ind. m^{-2}; 2018–2019) [20]. The population increase of the *Chironomus* taxa in the lakes deep zones is well documented, for example, in numerous lakes of Finland [47]. In fact, in August of the two years investigated, *C. plumosus* average larval density in the pelagic area amounted to about 755 ind. m^{-2} [20], equal to about four times the density of this taxon in the littoral population, that, on the other hand, represents only a small part of the Lake Trasimeno bottom, at most about 10%.

One of the primary purposes of this study concerns the analysis of the polyvoltine biological cycle of *C. plumosus* at Lake Trasimeno to highlight the number of its cyclical generations. The relative swarms of *C. plumosus*, attracted by the artificial lights, are particularly annoying in the summer, causing severe disturbances to the tourist and recreational activities of the coastal areas of the lake. This investigation was based on benthic samples (especially from the period 2017–2020), through the identification of the larval stages of *C. plumosus* (with our collection method, the 1st instar is impossible to detect due to its small size and its tendency to planktonic life [42]), and on the adult chironomid biomasses, essentially constituted of *C. plumosus*, captured from April to October (2018–2020) by the ChiroTraps.

The results show that *C. plumosus* overwinters as a mature larva in Lake Trasimeno; from January to March, only 4th instar larvae were found, with the rare presence of 3rd instar larvae. Only in April young larvae (2nd instar) were detected in relation to the first swarming of the year and the consequent first egg masses deposition. The spring emergence of *C. plumosus* has also been documented from Lake Suwa (Japan) in the study of Nakazato and Hirabayashi (1998) [49].

The examination of weekly benthic samples from May to October (2017–2020) showed that subsequently, considerable abundances of 2nd instar larvae were found, in particular at the beginning of July, mid-late August, and late September–mid-October, highlighting new egg masses depositions connected to relative swarmings (Table 4). In summary, during the year, from April to October, the presence of at least four *C. plumosus* generations can be highlighted. Similar results were obtained for *C. plumosus* from the shallow Zegrzyński dam reservoir (Poland), using similar methods based on frequent sampling and biometric parameter measurements of the larvae to determine the relative larval instar [50].

The results of the weekly collections of adult chironomids (in which *C. plumosus* was predominant), processed mainly through the data provided by the ChiroTrap located in Passignano sul Trasimeno (PA) in 2020, confirmed the aforementioned polyvoltine life cycle of *C. plumosus.* Four characteristic biomass peaks appear, corresponding to the four swarmings and lining up to the seasonal periods characterized by the presence of young larvae (Figure 4).

The number of biological cycles of insects is closely related to the environmental temperature. In 2019, in the 120 days from the last week of May (22nd week of the year) to the third week of September (38th), the water of Lake Trasimeno never fell below 20 °C, even at night. In experimental tests [51] conducted on the species *Chironomus riparius*, very similar to *C. plumosus* concerning size, morphology, and biological cycle, used in experimental tests [52], about 30 days are needed for the development of a larva from the 1st to the 4th instar, at a water temperature of 20 ± 1 °C. However, laboratory conditions are relatively suitable for the freshwater species under study, while field studies are influenced by various problems caused by biotic-abiotic factors [49,53]. In our tests, the time needed for the egg hatching, the transformation of larvae into pupae, and the subsequent swarming, needed about 10 additional days. Thus, the minimum amount for a complete biological cycle of *C. plumosus*, could be estimated at 800 degree-days (40 days at 20 °C = 800 degree-days); therefore, for the three summer cycles, 2400 degree-days are needed, and they are reached by the minimum condition of 120 days at 20 °C.

Based on the results of the present study, we can confirm that *C. plumosus* is a polyvoltine species [42,53], with at least four complete cycles under the current thermal conditions of Lake Trasimeno. In addition, it is logical to expect that the ongoing global warming process will tend to increase its number of cycles (generations) per year, with the relative consequences on the nuisance towards anthropic coastal activities. In relation to these perspectives, the present study provides a large amount of biological and environmental data for modeling the interventions aimed at containing the chironomid populations of Lake Trasimeno below the nuisance levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15010851/s1, Figure S1: Biometries of the head capsules of the *Chironomus plumosus* larvae from May to October on a weekly basis (2017–2020). 2nd instar larvae (x-axis, mean head capsule length: 0.26 mm; y-axis, mean head capsule width: 0.22 mm); 3rd instar larvae (0.53 mm; 0.42 mm); 4th instar larvae (0.95 mm; 0.79 mm); Table S1: Monthly mean density (2005–2021) of the eighteen Chironomidae taxa inhabiting Lake Trasimeno littoral zone (CA, Castiglione del Lago); Table S2: Presence in the months from 2005 to 2021 of the eighteen Chironomidae taxa inhabiting Lake Trasimeno littoral zone (CA, Castiglione del Lago).

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