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Leptoconops (*Holoconops*) *kerteszi* Kieffer (Diptera Ceratopogonidae) in the coastal area of Grosseto: eco-ethological aspects

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Abstract

Multi-year observations on the life-cycle of *Leptoconops* (*Holoconops*) *kerteszi* Kieffer (Diptera Ceratopogonidae) were carried out in the coastal areas of Grosseto (Italy). In the studied environment, most of the populations complete two generations within a year. Overwintering is assured by the larvae. Using a simple and functional sampling technique of preimaginal stages, we obtained a general overview of *L.* (*H.*) *kerteszi* breeding sites. *L.* (*H.*) *kerteszi* is generally found in coastal swampy areas, where the species colonizes a sandy shore that is devoid of vegetation and remains constantly damp as saltwater ascends by capillarity. Larvae living in the first centimeter of the shallow sandy layer. Such sites occur in interdunal sandy areas bordering the ponds closest to the seashore and also in tongues of sand along the edges of the ponds beyond the dunes, a little further inland.

Key words: *Leptoconops* (*Holoconops*) *kerteszi*, life cycle, breeding sites, insect ecology.

Introduction

The overwhelming majority of blood-sucking species of Diptera Ceratopogonidae belong to the genera *Culicoides* Latreille and *Leptoconops* Skuse. These species are among the most troublesome in the entire world (Kettle, 1962; Kettle, 1990; Linley, 1976; Süss, 1998). Kettle (1962) reported that the blood-sucking habit is limited to females, whose bites can transmit pathogens both to humans and animals. Furthermore, given to their high density, these Dipterans can cause considerable nuisance to people. They are distributed over wide areas, but species of the genus *Leptoconops* and many of the *Culicoides* live in close association with swampy coastal areas characterized by silty-clayey and sandy soils that rich in ponds of salt water. Such areas constitute the developmental environment of the preimaginal instars, which are linked to the water-soil ecotone (Kettle, 1962).

The coast near Grosseto, included in the Maremma Natural Reserve and in particular the coast from Marina di Alberese to Principina a Mare, represents one of the most distinctive and interesting marshlands of the whole Italian coastline. It is rich in saltwater ponds that are found not only in the sandy expanses that reach almost down to the shoreline but also a little further inland, beyond the line of dunes. In various other parts of the coastal area, such as along the banks of the River Ombrone and right up to its estuary, there is a predominance of clayey-silty soils, which are submerged for most of the year but dry out and crack in summer. The extension of these pools of standing water and their degree of salinity are strongly affected by rainfall, river flooding and, in the areas closest to the seashore, by tides and storm surges. Such contexts that are densely populated by wildlife, above all birds, as well as livestock and horses reared on the numerous local farms and allowed to graze freely on the range, form the ideal breeding ground for numerous species of Ceratopogonids of the genera *Leptoconops* and *Culicoides*.

In Italy the aggressive and irritant nature of *Leptoconops* species has long been known for a long time (Noè, 1907); females are typically found biting during the day in bright sunshine and often occur in such numbers. Although the presence of these Diptera has been globally reduced by the sweeping hydrogeological transformations following land reclamation campaigns, there still remain a number of marshy coastal areas that continue to be affected (Coluzzi, 1967; Bettini and Finizio, 1968; Moleas *et al*., 1998). In Tuscany, the problem is most troublesome in the coastal strip near Grosseto, with severe repercussions on health, hygiene, economic activity and tourism (Majori and Bettini, 1971). Five *Leptoconops* species have been recorded in Italy (Boorman, 1995), and all of them are present in Grosseto district: *Leptoconops* (*Holoconops*) *kerteszi* Kieffer, *Leptoconops* (*Leptoconops*) *irritans* Noè, *Leptoconops* (*Leptoconops*) *bezzii* Noè, *Leptoconops* (*Leptoconops*) *bidentatus* Gutsevich and *Leptoconops* (*Leptoconops*) *noei* Clastrier and Coluzzi. Among them, the most widely represented - and also the most bothersome in Grosseto coast - are *L.* (*H.*) *kerteszi* and in particular *L.* (*L.*) *irritans* (Cocchi *et al*., 1985).

This paper describes the biological cycle of *Leptoconops* (*Holoconops*) *kerteszi* Kieffer, and provides a detailed account of its breeding sites and the ecological niche occupied by the larval instars of this species in the coastal area of Grosseto.

Previous studies on larval reservoirs of this species have been carried out in the coastal area of Grosseto. Majori *et al*. (1970), using traps to capture newly emerged adults, identified some larval biotopes of *L.* (*H.*) *kerteszi* situated North and South of the mouth of the R. Ombrone, in sandy soil. Granulometric analysis by Majori *et al*. (1971) showed that these sites were characterized by elevated sand content. 93.01% - 98.54% of particles have a diameter ranging from 177 to 250 μm. Implementing the technique described by

Clastrier (1972) for recovery of preimaginal stages in soil, Cocchi *et al*. (1986a; 1986b) achieved a more precise identification of some larval biotopes.

Materials and methods

Sampling of preimaginal stages

The coastal area of Grosseto examined for this study on *L.* (*H.*) *kerteszi* extends from the Marina di Alberese up to the Principina a Mare beach, thus covering a stretch of coastline roughly 7 km long. Throughout this coastal strip numerous bodies of saltwater can be found. Some of them are temporary, others are fairly stable over time. In order to obtain information on the biology of the species under examination, observations and data logging were performed for two consecutive years (1998-1999). Weekly surveys were carried out during summer and fortnightly in winter. Observations were then continued at wider intervals in subsequent years up to 2004, in order to check that the findings were consistent with results obtained in the previous years. On the basis of data available in the specific literature (Smith and Lowe, 1948; Rioux and Descous, 1965; Foulk, 1966; Majori *et al*., 1970; Clastrier, 1972; Cocchi *et al*., 1986a; 1986b), we undertook the search for preimaginal stages of *L*. (*H*.) *kerteszi* by focusing mainly on the sandy shores of pools of water fairly close to the coast, up to a distance of roughly two hundred meters from the seashore. Fairly stable interdunal ponds as well as pools of standing water recently formed by the dynamicity of the coastline were examined. In addition, more stable ponds beyond the dunes, further away from the shoreline, were sampled.

Larval breeding sites were identified by means of a simple methodology using a kitchen spoon and a white plastic plate. Sampling was performed by taking small spoonfuls of sand (roughly 2 cm^3) at the following depths from the surface: up to 1cm, between 1 and 5 cm, and between 5 and 10 cm. The extracted sand was placed on the plastic plate with 10 ml of water taken from near the same area. The mixture was then shaken in order to raise the larvae and pupae from the current thus created and enable them to be deposited on the parts free from sand. With this technique the larvae, which were salmon colored, and the pupae, which were of a light brown color in the cephalo-thoracic part, were clearly visible against the white background, and were easily identified and removed with a Pasteur pipet. The material collected was immediately examined on site with the aid of a steroscopic microscope, and recorded. Ceratopogonid larvae and pupae were then transferred to the laboratory and reared to obtain adults.

Capture of adults

Females were captured on man by means of simple mouth aspirators. Samples were taken fortnightly starting from the period of the first appearance of specimens, which we identified as occurring around the end of March. Sampling was performed at pre-established distances from the shore, moving progressively closer to the seashore, such a sampling continued until the end of October. In parallel with aspirator captures, additional sampling was carried out by placing three chromotropic traps on the soil surface. The traps were composed of simple yellow plates filled with water and surface tension agents, addition of the latter being necessary to induce immediate sinking of adult specimens that were attracted by the yellow colour and settled on the liquid surface.

Traps were placed at progressively greater distances from the seashore: the first on the beach, just before the band of vegetation, the second within this band, and the third in the clayey-silty zone beyond the band of vegetation. These traps also allowed to capture males which are rarely recovered on man skin (Bettini *et al*. 1969b). Adults were preserved in 70% ethanol. Some specimens were clarified in potassium carbonate and mounted on slides, in Faure liquid, for identification at species level.

Results

Laboratory data

In the lab, fourth instar larvae that had been collected in the field and placed in petri dishes filled with moist sand from the sampling site were successfully reared to the stage of pupa and adult. The larvae are salmon colored and have an average length of 4 mm at maturity. They move with a snake-like motion, twisting their way between grains of moist sand. When placed in water they tend to curl up and display great difficulty in moving. The pupae measure roughly 1.5-2 mm and are light brown, with the abdomen pale yellow. The pupae are located close to the surface, almost completely buried in moist sand. They tunnel out from the sand only shortly before emergence, by moving the abdomen so that the cephalo-thorax and part of the abdomen protrudes.

In contrast to findings by Clastrier for France (1972; 1973a; 1973b and 1975), the numerous larvae and pupae collected in the field were morphologically homogeneous and, when reared in the laboratory gave adults (figure 1) all belonging to a single species of the subgenus *Holoconops*. We identified this as *kerteszi* Kieffer, according to the description of female (Kieffer, 1908); male terminalia were consistent with the description given by Coluzzi (1967) for this species.

Roughly 50% of females that emerged in the laboratory and survived few days in the petri dishes showed a fairly elevated number of ripe eggs, oval-arched in shape. This finding confirms the autogenic capacity of this species (Majori *et al*., 1970; Cocchi *et al*.,1986a). In contrast the females caught biting on man never showed mature eggs.

Breeding sites and ecological niche

The simple and functional sampling technique adopted in our approach allowed the recovery of an high number of preimaginal instars at each sampling and made it possible to extend rapidly sampling activity to additional areas. We were thus able to draw up a general criterion for non-ambiguous identification of the larval breeding biotopes and the ecological niche colonized by this spe-

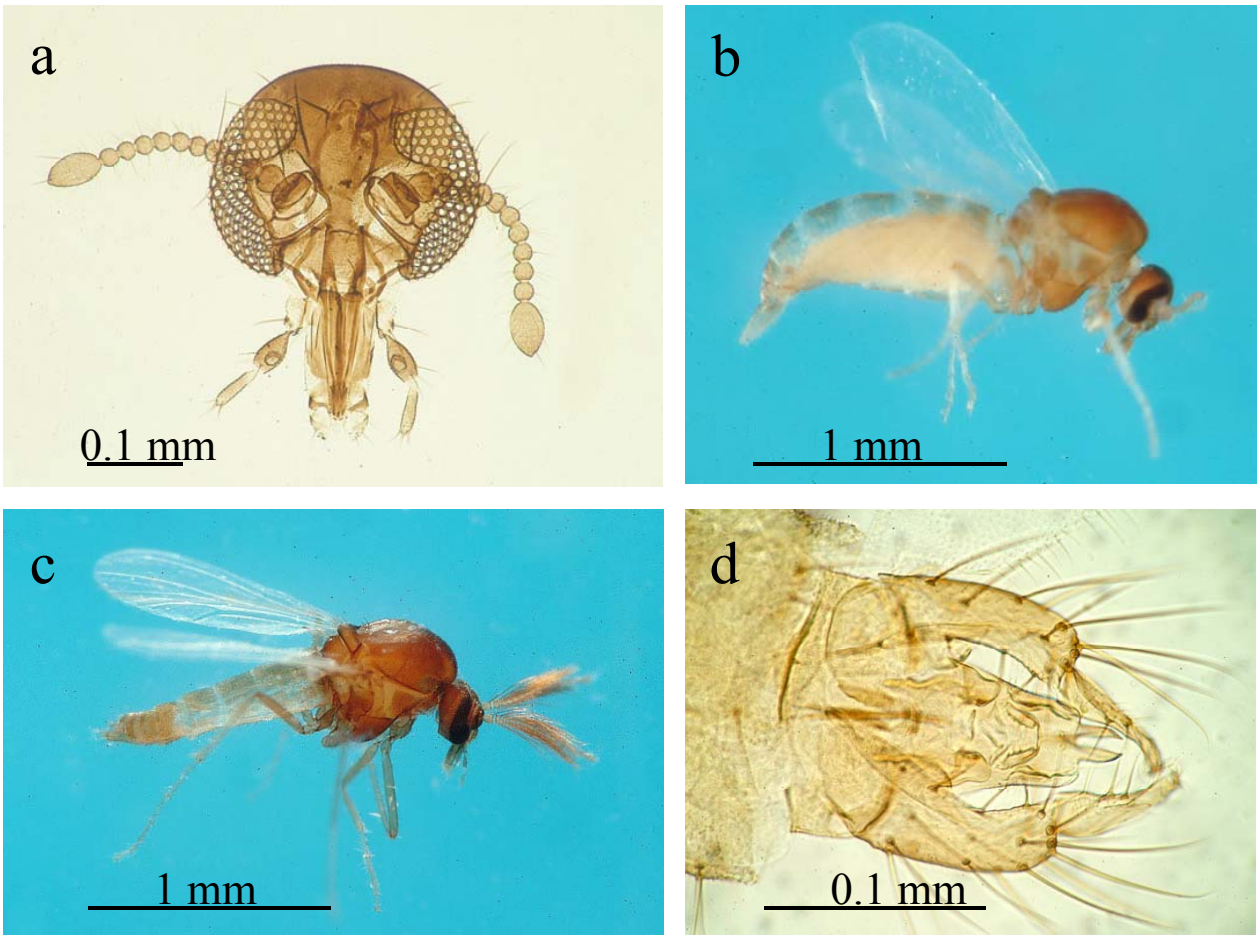

Figure 1. *L.* (*H.*) *kerteszi*: a) female head in frontal view; b) female and c) male preserved in ethanol; d) male terminalia.
(In colour at www.bulletinofinsectology.org).

cies. Our observations on sites where *L.* (*H.*) *kerteszi* larvae and pupae were recovered indicate that this species lives, along the coast, in sandy shores of ponds that are devoid of vegetation and are kept constantly moist by the capillary ascent of saltwater. The presence of this species seems to be favoured by a very shallow slope of the pond shore, which allows formation of a larger surface suitable for larval development (figure 2). Larvae can also be found in flattish sandy hollows even at considerable distance from the body of water provided that the underground reserve of moisture assures that the sandy bottom has elevated humidity. Other breeding sites of this species are tongues of sand along the gently sloping vegetation-free edges facing the dunes, of temporary or permanent pools of standing water, situated beyond the dunes with a predominantly clayey-silty bottom.

Larvae were found in the first centimeter of the shallow sandy layer, with a density up to 40 individual per cm^3. By contrast, negative results were obtained in the two deeper layers. Therefore the ecological niche occupied by these larvae is represented only by the shallow layer of moist sandy soils, up to a distance of 7-8 meters from the shore of the pond, as a function of moisture in the sand assured by the capillary ascent of saltwater. With increasing distance from the shore towards less moist sand, a relative increase in presence of pupal stages as compared to larvae was recorded.

Figure 2. *L.* (*H*) *kerteszi*: a typical breeding site. The arrow indicates moist sandy shore sloping gently towards saltwater where are present larvae and pupae.
(In colour at www.bulletinofinsectology.org).

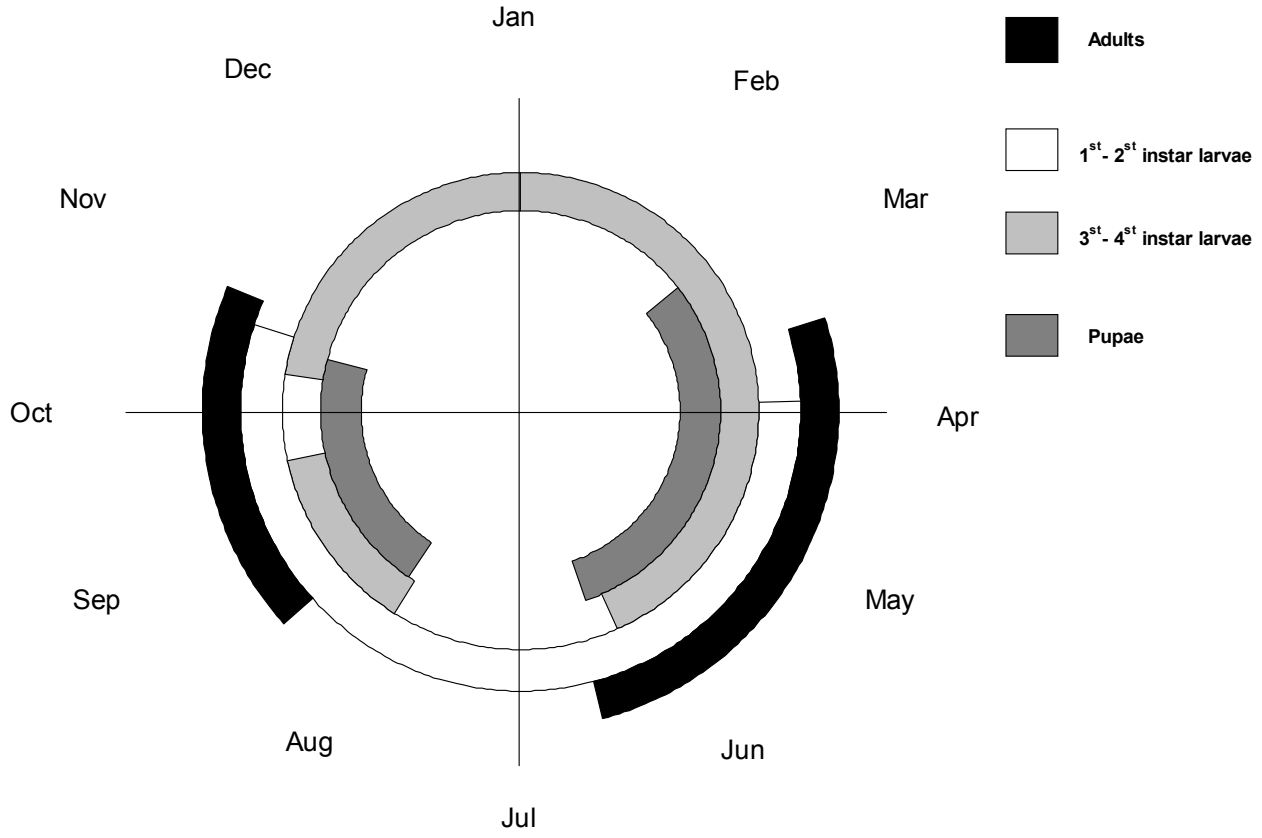

Figure 3. *L.* (*H.*) *kerteszi*: life cycle.

Biological cycle

Evidence of two flights during the course of a solar year was given by two particularly abundant periods of mouth aspiration captures on man. These results were then confirmed by laboratory emergences obtained from pupae collected in the field. The first flight occurred between late March and the beginning of June, the second occurred at the end of August, with captures continuing until the end of October (figure 3). During the period intervening between the two above-mentioned flight periods only a small number of adults were captured, using the chromotropic traps (yellow plates). Capture locations showed a marked preponderance of adult presences in zones stretching from the seashore up to the first vegetation beyond the dunes. This was confirmed by the trend of captures obtained only with the chromotropic traps, placed among the vegetation just beyond the beach and on the beach itself.

The periodic sampling of preimaginal stages, showed that overwintering occurred during the larval stage, with large numbers of larvae being found continuously at the sampling sites. The first pupae were found as early as the first half of March, and both pupae and larvae were found to be present concurrently until the end of May. In the first half of July third and fourth instar larvae reappeared, followed by pupae towards the end of the month and new adults between late August and early September.

Natural enemies

During spring the high density of *L.* (*H.*) *kerteszi* larvae and pupae represents a ready and easily locatable resource on which a complex trophic chain can be established. Direct observations revealed intense predation by adults of Diptera Dolichopodidae, *Thinophilus flavipalpis* (Zetterstedt) and *Tachytrechus ripicola* Loew, and Muscidae, *Lispe litorea* Fallén and *Lispe caesia* Meigen, on shores rich in larvae and pupae of *L.* (*H.*) *kerteszi*. As well as extensive copresence, in the sand samples observed, of larvae belonging to the genus *Lispe* Latreille, whose predation activity continued in the laboratory.

Discussion

Some larval biotopes of *L.* (*H.*) *kerteszi* were identified in previous studies by Cocchi *et al*. (1986a and 1986b) in areas examined in the present study. However, our sampling method allowed the inspection of entire area, leading to a precise and generalizable conclusion on the species breeding sites and the ecological niche occupied by preimaginal stages. With regard to this niche, our findings do not fully support reports by previous authors. In our observations, all larvae and pupae were found in the shallowest layer, within the first centimeter of moist sand. Thus previously reported depths of 0-5

cm (Clastrier, 1972) and 0-10, 10-20, 20-30 cm (Cocchi et al., 1982) were probably excessive, and may have been due to intrinsic defects of the sampling methods utilized for field sampling of larvae. Our findings are in accordance with the evidence that only the shallowest layer of moist sand, penetrated by light, has an abundant presence of unicellular algae and bacterial and fungal microflora on which *Leptoconops* larvae feed (Davies and Linley, 1965; Clastrier, 1972; Linley, 1976).

Analysis of the overall data obtained from the entire study period allowed to clarify some poorly understood aspects of the biology of this species, in particular with regard to the number of generations completed within the geographic area considered. Overwintering is assured mainly by third and fourth instar larvae, which, in contrast to pupae, are able to survive prolonged periods of water submersion. The larvae are able to move through the shallow layers of sand after rainfall. This explains why a greater presence of pupal stages of *L.* (*H.*) *kerteszi* is observed with increasing distance from the pond shore. These pupae give rise to the adults which emerge between the end of March and the month of May. Our field and laboratory data, confirming findings by other authors (Majori *et al*., 1970; Cocchi *et. al*., 1986b), demonstrate that depositions by adults emerged from mid March onwards give rise to an *L.* (*H.*) *kerteszi* population which succeeds, for the most part, in completing a generation between late spring and the beginning of autumn. This first generation produces adults captured in sampling between September and October, which give rise to the second generation. However, there is some evidence suggesting that a part of the population, the population deriving from delayed summer depositions, completes only one generation during the year, thus augmenting the overwintering larval population, which was particularly numerous. Considering the above-mentioned naturally scalar character of the biological cycle, it is not surprising that adults and in particular females with ripe eggs were captured, albeit in limited numbers, by chromotropic traps from late June to mid July. Such captures took place in a period when *L.* (*H.*) *kerteszi* was no longer recoverable on man, and in concomitance with extremely aggressive and massive attacks by another species present in the area, *L.* (*L.*) *irritans*.

L. (*H.*) *kerteszi* adults were recorded in the province of Ferrara and Ravenna (Coluzzi, 1967). These adults were captured massively on man between July and mid August, in contrast to findings for the Grosseto environment. The Ferrara and Ravenna captures can probably be attributed to different climatic trends and perhaps to lack of competition by other species such as *L.* (*L.*) *irritans,* which interferes with *L.* (*H.*) *kerteszi* in the Grosseto environment.

Our observations also provide further confirmation of the data reported by Davies (1965) concerning the environments frequented by *Leptoconops* adults. The *L.* (*H.*) *kerteszi* adults remain near the deposition sites and resting in the surrounding vegetation on windy days (Bettini *et al*., 1969b). Wind acts as a factor that disrupts the activity of adults, and under strong winds adults cluster in herbaceous and bushy vegetation. We also noted that in the periods of April-May and September-October the area where the presence of *L.* (*H.*) *kerteszi* was clearly predominant, was the zone stretching from the seashore up to the first vegetation beyond the dunes. In contrast, further inland a greater presence of *L.* (*L.*) *irritans* was found, as recorded by chromotropic trap captures and by the noticeably greater biting activity affecting man. *L.* (*L.*) *irritans* exhibits only one flight period (Bettini *et al*., 1969a; 1969b). During our observations, we captured this species from mid May to the end of August.

Conclusions

The simple and functional preimaginal sampling technique used in this study makes it possible to give an overall picture of *L.* (*H.*) *kerteszi* breeding sites. This species was found in moist sandy soils devoid of vegetation, situated in coastal marshy areas close to the sea. Larvae and the pupae were recovered in the first centimeter of the shallowest sandy layer composed of fine sand and organic matter. Three different typologies of breeding sites were identified:

- moist sandy shores, devoid of vegetation, sloping gently towards saltwater, bordering bodies of water that may be fairly large and which vary considerably in surface area during the summer season, leaving exposed lengthy stretches of moist sand on which the larvae develop;
- sandy hollows that may lie at some distance from the edge of the water but which remain moist due to the humidity present beneath the soil, as they are situated not far above the water table; the center of such hollows may be temporarily under water after heavy rainfall or due to the penetration of seawater;
- tongues of sand along the gently sloping vegetation-free edges facing the dunes of temporary or permanent pools of standing water, situated beyond the dunes with a predominantly clayey-silty bottom.

These sites can be found both in sandy areas among the dunes close to the ponds that are nearest to the seashore, and also on the sandy edge of ponds further inland beyond the dunes. Sites near the shore seem to play an important role in sustaining the species by providing a fairly vast habitat that is constantly present, even though it is subject to dynamic variation. Sites beyond the dunes play a less important role due to a more limited presence of sandy areas suitable for development of larval forms. On the other hand, pools of water beyond the dunes are extremely important as a reservoir for conservation of the species during periods when pools closer to the seashore disappear as a result of coastal disturbance and evolution of the sand coastline. In this regard, it should be noted that the Grosseto coast is characterized by marked dynamicity and is frequently affected by drastic variations.

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