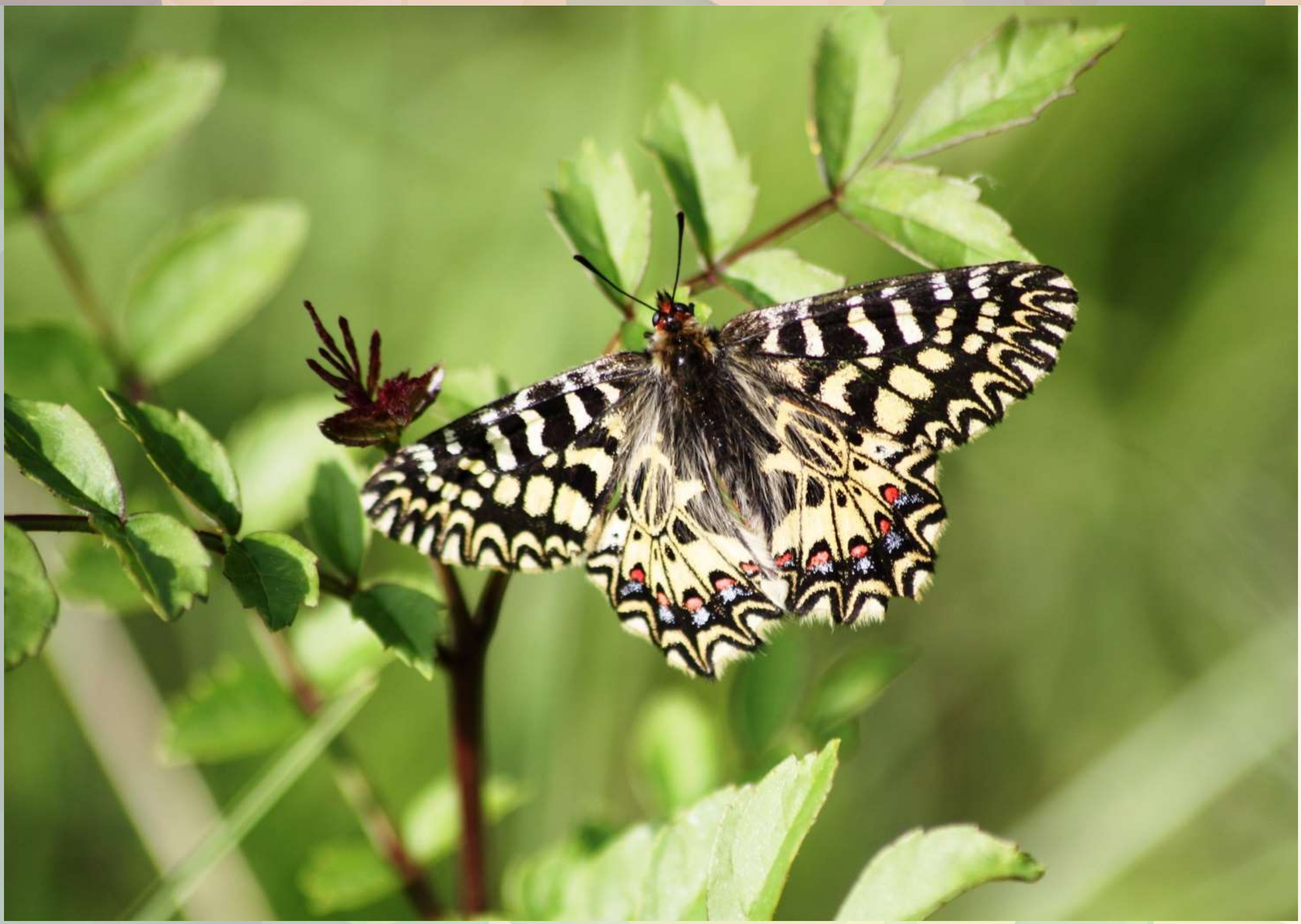
Monitoring of the population of the Southern Festoon (*Zerynthia polyxena*) and preliminary assessment of the effect of late mowing on birthworts (*Aristolochia*)

Marais de l'Étroit, Vallée des Baux
Bouches-du-Rhône, France



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INTRODUCTION

The Southern Festoon (*Zerynthia polyxena*), Diane in French, was first described in 1775 by Denis & Schiffermüller. This species is interdependent with a plant, the birthwort (*Aristolochia)*. Developing in open environments, this plant today has a very fragmented habitat. As a result, most populations of Southern Festoon are very small and very localised. The first large-scale study was only carried out in 2012 by Celik *et al.* in Slovenia on 8.7 hectares of continuous favourable environments. This last study highlighted a decline of about 30% in the studied Southern Festoon population over the last 10 years. Today this butterfly is classified as a threatened species. It is listed in Annex IV of the Habitat-Fauna-Flora Directive, in Annex II of the Bern Convention and is protected at national level (INPN). Its presence on a site must therefore be subject to appropriate management for the preservation of the species. As the distribution of this butterfly is very local, and linked to the distribution of its host plant, the birthwort, its protection is all the more targeted. The management plan can therefore be very site-specific, on a small plot of land, which makes it easy to implement while remaining very effective. This was achieved in 2012 for the population present at the marsh of l'Étroit by the association A Rocha France. The management plan put in place recommends mowing the vegetation during the Southern Festoon's hibernation to open up the site without harming the individuals present. Indeed, the interest here is to allow the population of birthworts to increase and thus maintain, or even expand, the habitat favourable to the Southern Festoon, in order to extend its range. Several monitoring programmes have been set up within the framework of this management plan in order to monitor the populations and evaluate the management implemented. In particular, it was planned to monitor the Southern Festoon population by Capture-Marking-Recapture (CMR) as well as the birthworts by counting the number of individual plants on quadrats distributed according to the implemented management.

The present report details and analyses the results of the studies carried out in 2013 and 2015, and in particular two censuses of the Southern Festoon population conducted by CMR and which resulted in an administrative exemption authorising capture.

1. EQUIPMENT AND METHODS

1.1 The Southern Festoon

The Southern Festoon is a rhopalocera of the family *Papillonidae*, which can be found from south-east France to Asia Minor (Figure 1). The distribution of this species is dependent on its host plant which belongs to the genus *Aristoloches*. With a yellow upperside decorated with black, a few red spots on the forewings, and a wingspan of 46 to 62 mm, this butterfly is easily recognisable and is unmistakable. Its flight period is very short, from March to May; it then hibernates by forming a pupa in the surrounding vegetation (Figure 2).

Its habitat is limited to the sites colonised by the birthwort. It is under the leaves of this plant that Southern Festoon lays her eggs, which the caterpillars feed on. It has been observed by Celik (2012) that all the individuals are confined to sunlit birthwort. The males occupy a smaller territory than the females. This is similar to the reproduction strategy chosen by the female: spreading the eggs over a large territory and looking for a partner during the reproduction period. The birthwort is included in all the life cycles of the Southern Festoon, except for the chrysalis state, which takes place on higher vegetation such as a tree branch. This plant develops mainly in humid meadows, but can be observed in drier environments (scrub, crops, dry lawns, rocky steppes) or in wasteland, orchards, vineyards, sparse deciduous woods. It is crucial to consider the needs and extent of this species in the study of the Southern Festoon butterfly.

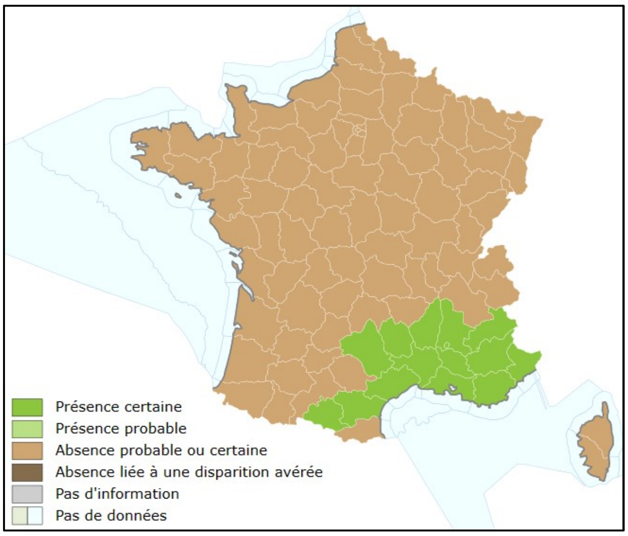


Figure 1: Distribution of the Southern Festoon in France (*Source: http://inpn.mnhn.fr*)

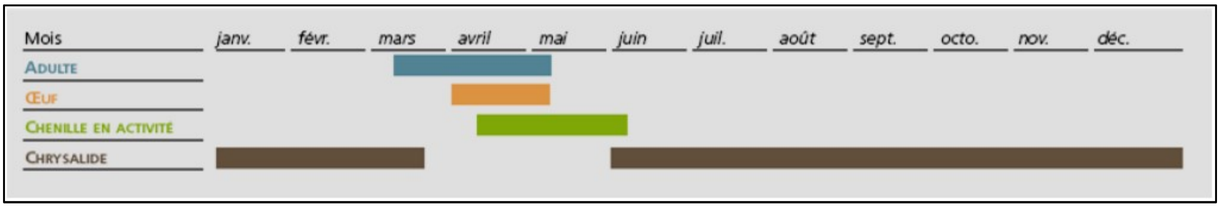


Figure 2: Life-cycle of the Southern Festoon (*Source : http://ecologie.gouv.fr*)

1.2. The study site

The Etroit marshland is located in the Baux valley, in the communes of Paradou and Maussane. It is a small wetland of 6.6 hectares, located between the Ilon marshland and the Pont de l'Etroit bridge. This study is only concerned with the central meadow of the Étroit marshland. Indeed, because of the presence of Round-leaved birthwort (*Aristolochia rotunda*) and European birthwort (*Aristolochia clematitis*), the latter is home to a population of Southern Festoon. As the Marais de l'Etroit is both in the Alpilles Regional Natural Park and in the Natura 2000 site of the Special Area of Conservation "Marais de la vallée des Baux et Marais d'Arles", this meadow is part of a group of protected sites. The map on the following page (Figure 3) represents the meadow and the different ecological units that make it up. It can be seen that the site is mainly made up of a wet meadow, a favourable environment for the development of birthworts. A trend towards the reduction of the environment with the colonisation of woodland was observed from 2008 to 2012, following the cessation of grazing on the site by a herd of goats. This observation led to the drafting of a management plan by A Rocha France and a late mowing of the vegetation was implemented to favour the birthwort.

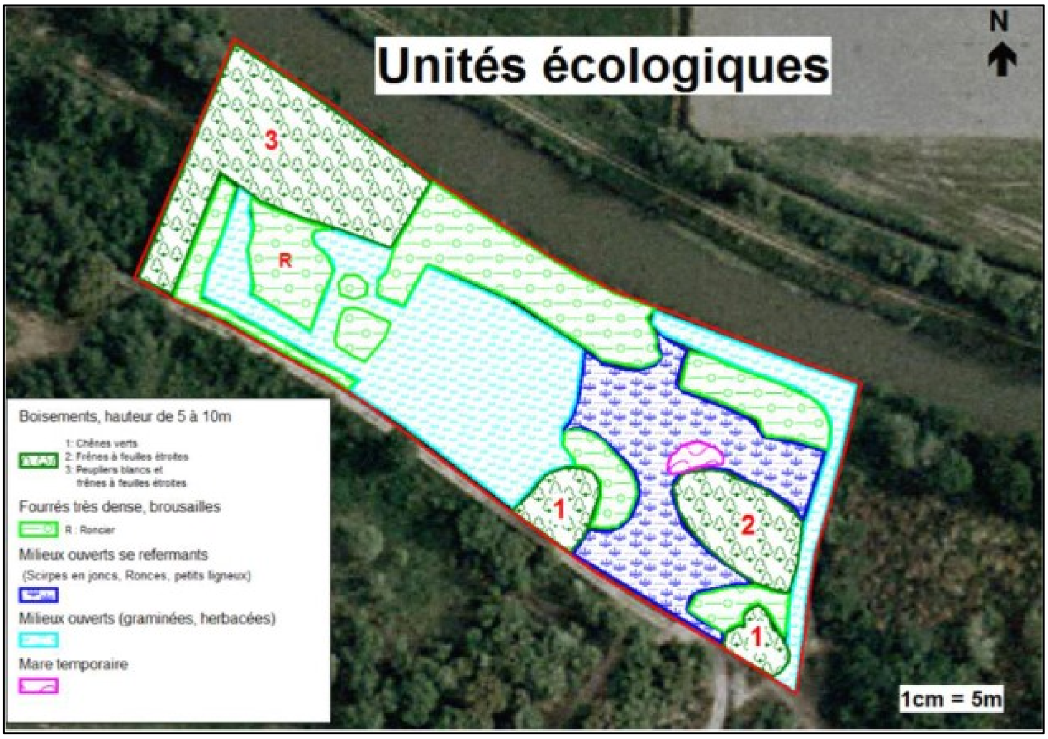


Figure 3: Ecological units of the central meadow of the Etroit Marsh (*Source: A Rocha: Management plan for the central meadow of the marais de l'Étroit, 2012 to 2017)*

1.3. Southern Festoon population count

1.3.1. Protocol applied

To estimate the size of the Southern Festoon population, A Rocha applied the Capture-Mark-Recapture (CMR) method. This method makes it possible to estimate the size of a population and to monitor its evolution over time. The protocol begins when pupae hatch (in March) and ends when no more individuals are encountered (end of May). The entire meadow is criss-crossed according to a defined route (Figure 5) three times a day, three days a week (and depending on the weather conditions). Sessions start at 10am, 12pm, and 5pm to allow for differentiation between the probability of observation and the probability of presence. Each Southern Festoon encountered is captured and marked on the wings with a coloured alcohol marker, according to a specific code (Figure 4) enabling it to be recognised (see Appendix 1), then released. A unique number is then assigned to each captured butterfly, allowing a second observation to identify the individual in question. For example, the third butterfly contacted will bear the wing mark corresponding to the number 3, which will then become its identifier. Each marked butterfly and each recaptured butterfly is listed binomially (1 when captured/recaptured, 0 when not observed) on the field sheet. If an individual is observed several times during the same session, it is counted only

Figure 4: Example of a captured and marked Southern Festoon *(©A Rocha France)*

once. The weather should be suitable for butterfly observation: sunny with light/no wind. The associated variables recorded are the following: date, temperature, wind (strength and direction), cloud cover, observer and any changes or remarks on the weather during the day.

Figure 5: Circuit taken for the CMR on the meadow of the Entroit Marsh (*Source : A Rocha: Management plan for the central meadow of the marais de l'Étroit, 2012 to 2017)*

1.3.2. Statistical analysis

The CMR data were analysed with the MARK software. With this software, it is possible to estimate the size of a population, the number of individuals present at each session, the probability of survival of individuals, the probability of capture, while including associated variables (time, age). From the monitoring sheets, the capture histories of each individual captured at least once were recorded in a spreadsheet. For each of the two monitoring years (2013 and 2015), the capture stories of each individual were summarised by day ("1" if the individual was seen at least once during the 3 sessions, "0" otherwise). The suitability of a full CJS model with time-dependent probability of survival and capture was first tested using Fletcher's c-hat. A selection of models was conducted by modelling all possible combinations of the probability of capture and the probability of survival (constant " . "; random as a function of day "t"; evolving linearly "tlin" or quadratically "tlin+tlin^2" as a function of time), in all 16 models. The best models were selected on the basis of the AICc (ΔAICc<2). This data set was then analysed using the "POPAN" module of the MARK software. This module makes it possible to estimate the population size at each session (day in this case) as well as the size of the total population, taking into account the arrival of new individuals between sessions, linked to the progressive metamorphosis of the nymphs present on the site. A model selection was carried out on the probability of entering the population (constant " . "; random as a function of day "t"; evolving in a linear "tlin" or quadratic "tlin²" fashion as a function of time) by considering the best CJS model for the structure of the probability of survival and the probability of capture.

1.4. Study of the effect of management on density in birthworts

This study was conducted in April 2014. The aim was to evaluate the effect of mowing on the Southern Festoon's host plant, the Round-leaved birthwort, at the site of the marsh of l'Etroit. To do this, the study consisted of estimating the density of birthworts (*Aristolochia rotunda* and *Aristolochia clematitis*) by means of exhaustive counts within 1m² (1m x 1m) quadrats. 62 quadrats were distributed homogeneously over the study area and an adjacent sector that benefited from different management (equine grazing) (Figure 7). For each quadrat, the number of birthworts of each species was counted and reported on a field sheet. The type of management (mowing, no management, grazing) and the exposure of the quadrat (sunny or shaded) were also recorded. The analysis of the results consisted in comparing the density of birthworts according to the type of management. We tested the hypothesis that mowing favoured the density of birthworts using a generalised linear model with an additive effect of the type of management and exposure as explanatory variables and the number of birthworts as response variable, with a binomial negative distribution law to take into account over-dispersion. The analysis was carried out for each species of birthwort separately as well as for the total number of birthworts. These statistical analyses were carried out using the R software.

Figure 6: Round-leaved birthwort
(© A Rocha France)

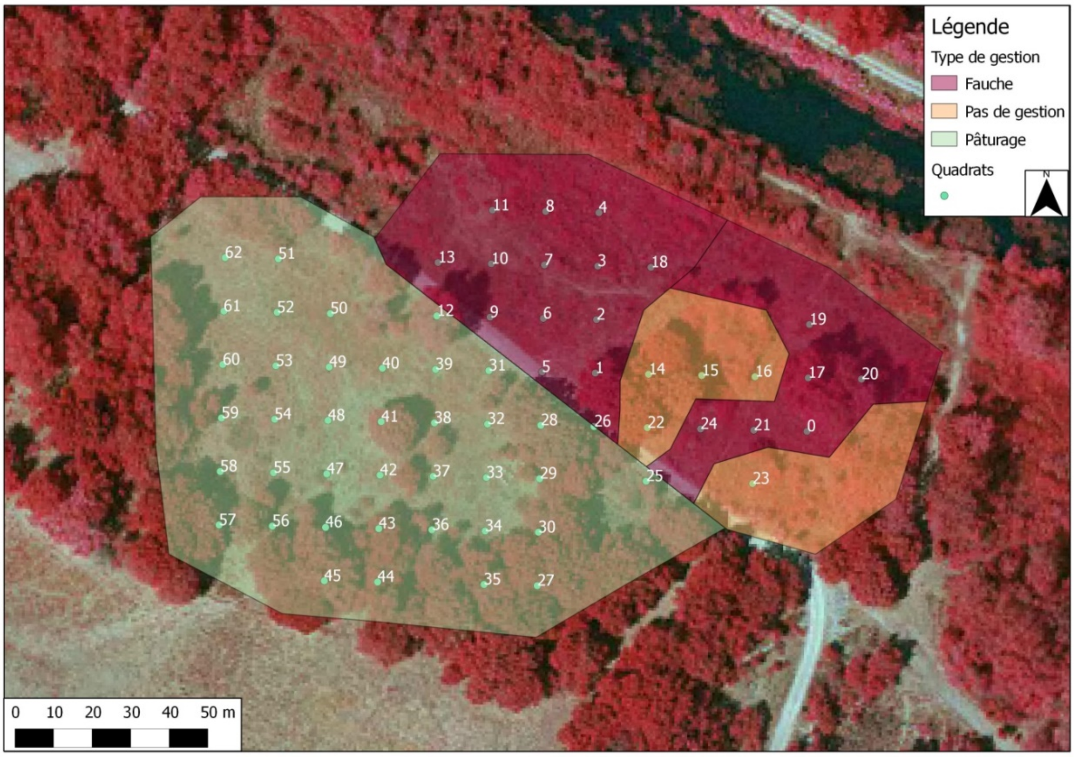


Figure 7: Location of the centres of the quadrats carried out for counting birthworts (*Aristolochia rotunda* and *Aristolochia clematitis*), in April 2014, according to the different type of vegetation management (mowed, not managed, grazed)**.**

2. RESULTS

2.1. Southern Festoon population count, 2013

In 2013 we carried out 42 capture sessions spread over 14 days (3 sessions per day) between 3 April and 3 May 2013 (Table 1 and Appendix 2) and marked 90 different Southern Festoons. The largest number of different individuals was observed on 16 April 2013 (n=35) (Figure 8). The majority of the catches took place at noon (Table 2).

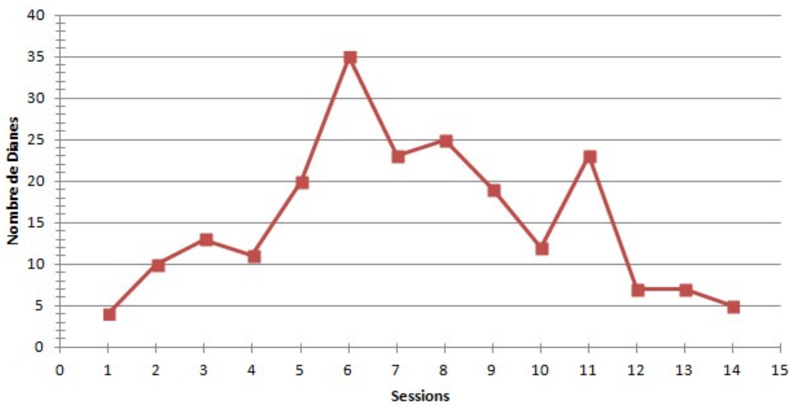


Figure 8 : Number of Southern Festoons observed on different capture days in 2013

The best CJS model based on the AICc is the {*Phi(tlin) p(.)*} model (Table 1).

This model gives an average recapture probability of 0.48 [0.38;0.58].

The best model giving an average survival probability is the {*Phi(.) p(.)*} model with an estimated daily survival of 0.84 [0.80;0.87].

Table 1: Selection of CJS models for the CMR data of Southern Festoons in the Etroit Marsh in 2013.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Phi(tlin) p(.)}	368.8687	0	0.37013	1	3	152.8117
{Phi(tlin+tlin²) p(.)}	370.2681	1.3994	0.18385	0.4967	4	152.1114
{Phi(tlin) p(tlin)}	370.5186	1.6499	0.16221	0.4383	4	152.362
{Phi(tlin+tlin²) p(tlin)}	371.6591	2.7904	0.09171	0.2478	5	151.3768
{Phi(.) p(.)}	372.3716	3.5029	0.06422	0.1735	2	158.3887
{Phi(tlin) p(tlin+tlin²)}	372.6425	3.7738	0.05609	0.1515	5	152.3601
{Phi(tlin+tlin²) p(tlin+tlin²)}	373.6651	4.7964	0.03364	0.0909	6	151.2304
{Phi(.) p(tlin)}	374.3822	5.5135	0.0235	0.0635	3	158.3252
{Phi(.) p(tlin+tlin²)}	376.0313	7.1626	0.0103	0.0278	4	157.8747
{Phi(tlin) p(t)}	379.0425	10.1738	0.00229	0.0062	15	135.9539
{Phi(tlin+tlin²) p(t)}	381.4786	12.6099	0.00068	0.0018	16	135.9421
{Phi(t) p(.)}	381.8175	12.9488	0.00057	0.0015	14	141.1447
{Phi(t) p(tlin)}	382.2865	13.4178	0.00045	0.0012	15	139.198
{Phi(.) p(t)}	383.7154	14.8467	0.00022	0.0006	14	143.0426
{Phi(t) p(tlin+tlin²)}	384.7086	15.8399	0.00013	0.0004	16	139.1723
{Phi(t) p(t)}	394.8995	26.0308	0	0	25	125.77

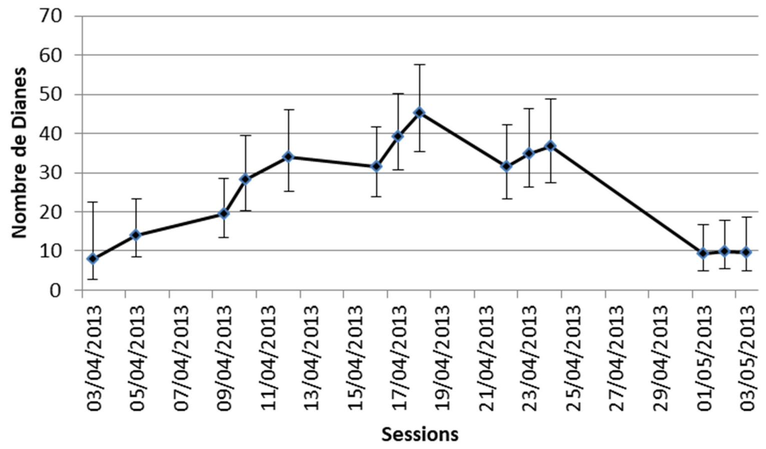
Table 2: Total captures by time of day in 2013

Time	Morning (10h)	Noon (14h)	Evening (17h)
Total number of Southern Festoon captured	79	94	43

The best POPAN model based on the AICc is the {*Phi(tlin) p(.) pent(tlin+tlin²)*} model.

This model gives an estimate of Southern Festoon's population size of 138 individuals [118;161]. It also indicates a maximum number present in the population during session 8, on 18/04/2013 (Figure 9).

Figure 9: Estimation of the Southern Festoon population present on each capture day in 2013, with its degree of confidence.



2.2. Southern Festoon population count, 2015

In 2015 we carried out 30 capture sessions spread over 10 days (3 sessions per day) between 29 March and 22 April 2015 (Table 3 and Appendix 3) and marked 41 different Southern Festoons. The largest number of different individuals was observed on 08 April 2015 (n=26) (Figure 10). The majority of catches took place in the morning (Table 4).

The best CJS model based on the AICc is the {*Phi(tlin) p(.)*} model (Table 2).

This model gives an estimated average recapture probability of 0.65 [0.48;0.79].

The best model giving an average survival probability is the {*Phi(.) p(tlin)*} model with an average daily survival probability of 0.86 [0.79;0.91].

Table 3: Selection of CJS models for the CMR data of Southern Festoons in the Etroit Marsh in 2015.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
{Phi(tlin)p(.)}	146.709	0	0.24937	1	3	53.0935
{Phi(.)p(tlin)}	147.1468	0.4378	0.20035	0.8034	3	53.5313
{Phi(tlin)p(tlin)}	147.3566	0.6476	0.1804	0.7234	4	51.5235
{Phi(tlin+tlin²)p(.)}	148.8706	2.1616	0.08462	0.3393	4	53.0375
{Phi(.)p(tlin+tlin²)}	149.0895	2.3805	0.07585	0.3042	4	53.2564
{Phi(tlin+tlin²)p(tlin)}	149.6023	2.8933	0.05869	0.2354	5	51.4918
{Phi(tlin)p(tlin+tlin²)}	149.6313	2.9223	0.05785	0.232	5	51.5208
{Phi(.)p(.)}	149.6395	2.9305	0.05761	0.231	2	58.1839
{Phi(tlin+tlin²)p(tlin+tlin²)}	151.9401	5.2311	0.01824	0.0731	6	51.4897
{Phi(t)p(tlin)}	153.6779	6.9689	0.00765	0.0307	8	48.3501
{Phi(tlin)p(t)}	154.6992	7.9902	0.00459	0.0184	9	46.828
{Phi(t)p(.)}	156.6915	9.9825	0.0017	0.0068	9	48.8203
{Phi(tlin+tlin²)p(t)}	157.3137	10.6047	0.00124	0.005	10	46.8256
{Phi(.)p(t)}	158.0231	11.3141	0.00087	0.0035	9	50.152
{Phi(t)p(tlin+tlin²)}	158.2393	11.5303	0.00078	0.0031	10	47.7512
{Phi(t)p(t)}	160.8939	14.1849	0.00021	0.0008	12	44.9375

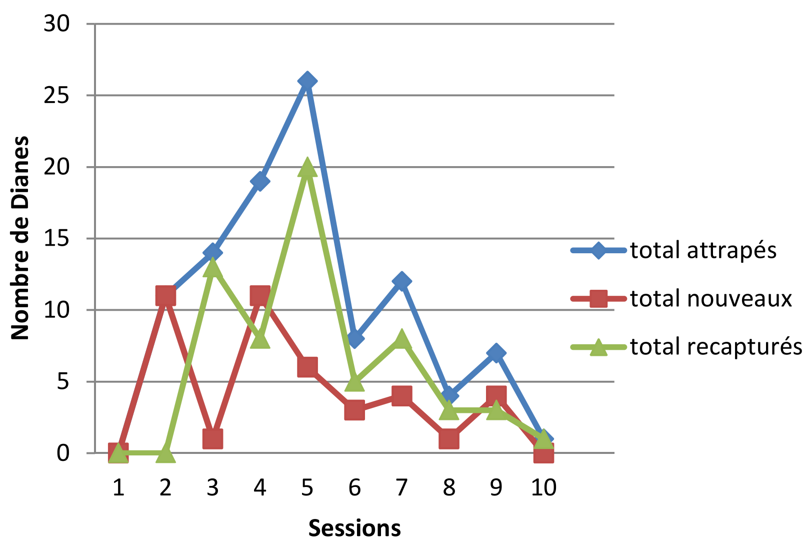


Figure 10: Total numbers caught by day of survey. (Blue: total caught; red: total new caught; green: total recaptured.)

Table 4: Total captures by time of day in 2015

Time	Morning (10h)	Noon (14h)	Evening (17h)
Total number of Southern Festoon captured	43	37	22

The best POPAN model based on the AICc is the {*Phi(tlin) p(.) pent(tlin)*} model.

This model gives an estimate of Southern Festoon's population size of 56 individuals [47;67]. It also indicates a maximum number present in the population during session 5, on 08/04/2015 (Figure 11).

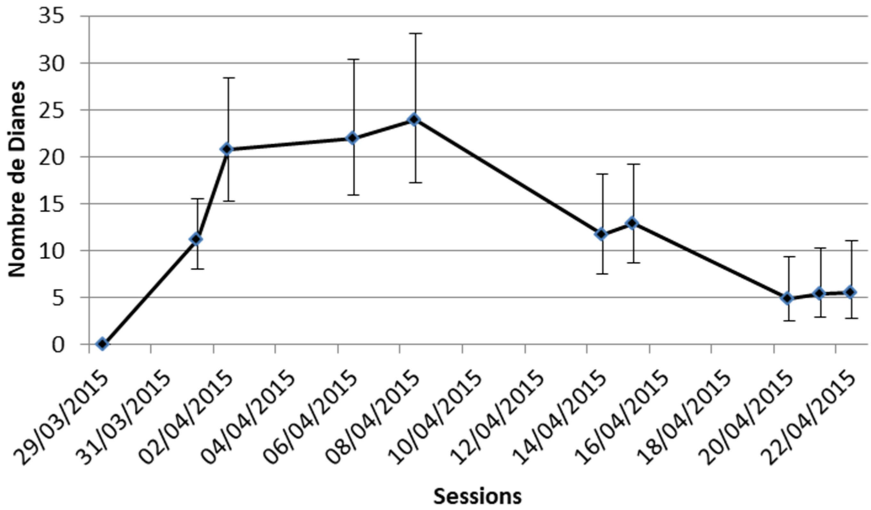


Figure 11: Estimation of the Southern Festoon population present on each capture day in 2015, with its degree of confidence.

2.3 Follow-up of the birthworts, 2014

426 birthwort individuals were recorded on the 62 quadrats in 2014 (respectively 285 *A. rotunda* and 141 *A. clematitis*), i.e. an average density of 6.9 feet per m^2 (respectively 4.6 for *A. rotunda* and 2.3 for *A. clematitis*).

The total density of birthworts was significantly higher on mowed than on grazed blocks (18.2 [6.8;48.3] vs. 1.2 [0.6;2.6], $p<0.01$) and more particularly for the round-leaved birthwort (11.6 [3.9;34.3] vs. 0.8 [0.3;1.9], $p<0.01$) but not for the *A. clematitis* (6.6 [0.7;60.0] vs. 0.4 [0.1;2.1], $p=0.05$) (Figure 12). Moreover, in the mowed area, the shaded quadrats have a significantly higher density than the exposed quadrats for the round-leaved birthwort (85.7 [15.7;467.4] vs. 10.9 [4.0;29.7], $p<0.01$).

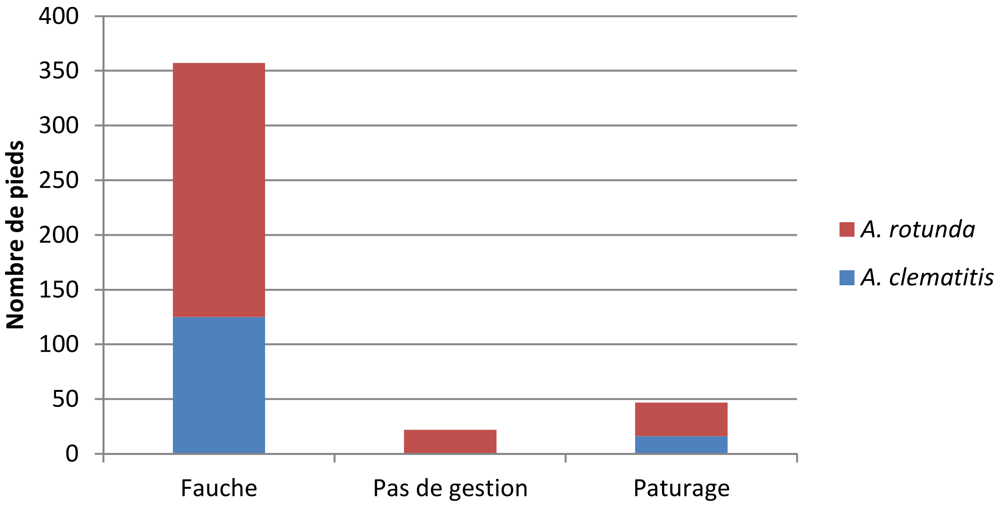


Figure 12: Total number of individual birthworts counted by type of management (mowed, no management, grazed)**.**

3. DISCUSSION

3.1. Southern Festoon population count

The estimated population size differs greatly between 2013 and 2015, with a significantly lower population in 2015. This result raises the question of an apparent decline in the population size of Southern Festoons at the study site. However, inter-annual fluctuations in butterfly numbers are known for many species (Stewart *et al*. 2020; Roy *et al*. 2015; Stefanescu, Peñuelas, and Filella 2003). In butterflies, these fluctuations are notably linked to interannual variations in climate, with for example a negative effect of high temperatures at the end of wintering on the survival of larvae, which can impact the size of the imago population (Radchuk, Turlure, and Schtickzelle 2013). Temperature variations can also decorrelate metamorphosis and availability of food resources and host plants (Davies 2019) or the emergence of male and female individuals (Bubová *et al.* 2016), which can have an effect on population dynamics in the medium term (Bubová et al. 2016; Davies 2019). Moreover, in our study, the peak abundance observed is clearly staggered between the two years of study, since it occurs on 18 April in 2013 and on 8 April in 2015. Among butterflies, it is known that weather conditions can influence the emergence dates of imagos (Stewart *et al*. 2020), and in particular the temperature during the month of March, with earlier emergence in warm years (Stewart *et al.* 2020). It is therefore likely that the differences in emergence dates observed between the two monitoring years are related to different weather conditions, which could explain the observed differences in numbers. It will be useful to consider the implementation of long-term monitoring of the population, allowing a comparison between years of population size and date of emergence of the imagos. Parallel monitoring of a population in an unmanaged control area would be necessary in order to be able to separate the effects of management and climate variation and thus be able to assess the effect of management on the population dynamics of Southern Festoons.

The probability of individual survival of the Southern Festoons in the study site is around 0.8 over the two years of monitoring. This average probability makes it possible to estimate an average lifespan of 4.5 days. However, the average life expectancy of individuals can be much longer: a marked butterfly was observed on 01/04/2015, then recaptured on 21/04/2015. These values are perfectly similar to those observed in Slovenia by Celik (2012).

The probability of recapturing individuals during one day of monitoring (i.e. 3 sessions) is relatively high and varies between 0.48 in 2013 and 0.65 in 2015. The study by Celik (2012) on a total of 162 tagged individuals obtained a recapture probability of 0.46. The protocol set up here therefore seems to be effective. Within the same day, captures are more numerous during the morning and midday sessions, which would make it possible to direct future prospecting towards these time slots: the favourable hours for the capture of the Southern Festoon seem to be between 10am and 4pm. Bringing the last passage forward to 3 pm could be envisaged. At 5pm, the site is less sunny and the butterflies are less active.

Other suggestions for future censuses are to carry out more intensive monitoring, with surveys every day rather than just three days a week. However, several factors may limit this frequency of monitoring, particularly weather conditions.

3.2. Monitoring of birthworts, 2014

Overall, our study revealed a high density of birthworts on the site, which is a positive point for the population of Southern Festoon present at the Marais de l'Etroit. The counting of the individuals of the Southern Festoon's host plants reveals a dominance of the species *A. rotunda* over *A. clematitis* on the meadow of the Marais de l'Etroit. The environment therefore seems to be more favourable to the development of *A. rotunda*, the species on which Southern Festoon carries out her entire reproductive cycle, in a privileged manner on the study site.

Mowed quadrats revealed a significantly higher density of birthworts than unmowed quadrats, particularly for *A. rotunda*. The mowing technique therefore seems to be favourable to the development of birthworts. However, *A. rotunda* was also more abundant on shaded quadrats. Thus, the maintenance of trees within or on the periphery of the site seems favourable to its development. These results will have to be consolidated by future studies, in particular by increasing the sample size on the different management methods.

CONCLUSION

This study highlights the presence of a large population of Southern Festoon on the Marais de l'Etroit site. *A. rotunda*, its host plant, is well established on the site, and management by late mowing seems to favour its development. However, the decrease in the size of the Southern Festoon population between the two years of monitoring raises questions and needs to be explored in the long term. The census protocol by CMR set up makes it possible to estimate the size of the population and to detect variations in numbers between years and within the same season. By continuing this study over several years, it will be possible to see the evolution of the population over time.

The meadow of the Etroit marsh is not the only site with a presence of the Southern Festoon in the Vallée des Baux, even if it undoubtedly hosts the finest population of the territory. It is likely that if the population of Southern Festoon on the site decreases, the links between this population and the other populations could become unstable and eventually lead to a decomposition of the metapopulation. This could lead to the local extinction of Southern Festoon. For this reason, maintaining or even expanding the area of wet grasslands is essential to maintain a healthy population of Southern Festoons (van Swaay *et al.* 2012).

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APPENDICES

Appendix 1: Marking code for Southern Festoons

Appendix 2: Description of the different CMR sessions in 2013

Sessions	Date	T°C	Clouds (/100)	Wind strength (km/h)	Wind direction	Observer(s)	Comments (weather etc)
A	**03-04-2013**	8°C/21°C	0-30	10	W	EW. VD	
B	**05-04-2013**	11°C/19°C	0-70	5	W	EW. VD	
C	**09-04-2013**	16°C/18°C	5-60	5	ENE	EW. VD	
D	**10-04-2013**	11°C/21°C	0-50	5	SW	EW. VD. CR	
E	**12-04-2013**	12°C/22°C	0-5	10	S	EW. VD	
F	**16-04-2013**	13°C/22°C	0-5	0	-	EW. JP	
G	**17-04-2013**	15°C/22°C	0-5	5	SE	EW. VD. DW	
H	**18-04-2013**	18°C/22°C	0	0	-	EW. VD. TF	
I	**22-04-2013**	16°C/20°C	0	20	W	EW. VD. CR	
J	**23-04-2013**	18°C/22°C	0-95	5	W	EW. VD. CR	
K	**24-04-2013**	18°C/22°C	0-20	5	S	EW. VD. CR	
L	**01-05-2013**	12°C/20°C	0-100	5	SE	EW. VD.	Heavy rain early in the morning
M	**02-05-2013**	16°C/17°C	0-100	5	-	EW. VD.	Misty; some ashes falling from a nearby fire
N	**03-05-2013**	17°C/18°C	0-50	10	N	EW. VD.	

Appendix 3: Description of the different CMR sessions in 2015

Sessions	Date	T°	Clouds (/100)	Wind strength (km/h)	Wind direction	Observer(s)	Comments (weather etc)
A	**29/03/2015**	17°C/20°C	35-45	29	NW	NM	
B	**01/04/2015**	12°C/15°C	15-25	44	NNW	NM	
C	**02/04/2015**	13°C/19°C	0-50	35	NNW	NM	
D	**06/04/2015**	12°C/16°C	0	27	N	NM	
E	**08/04/2015**	14°C/19°C	0	13	SW	NM	
F	**14/04/2015**	24°C/27°C	0	24	N	HC	Wind squalls
G	**15/04/2015**	16°C/22°C	0-20	25	S	HC	
H	**20/04/2015**	14°C/19°C	40-60	20	W	HC	
I	**21/04/2015**	17°C/22°C	0	15	S	HC	
J	**22/04/2015**	18°C/22°C	20-100	20	S	HC	
K	**06/05/2015**	22°C/25°C	0-10	35	NW	HC	0 Southern Festoons observed