

Accepted Manuscript



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PII: S1616-5047(15)00034-8
DOI: <http://dx.doi.org/doi:10.1016/j.mambio.2015.03.008>
Reference: MAMBIO 40748

To appear in:

Received date: 28-7-2014
Revised date: 27-3-2015
Accepted date: 27-3-2015

Please cite this article as: Puig-Montserrat, X., Torre, I., López-Baucells, A., Guerrieri, E., Monti, M., Ràfols-García, R., Ferrer, X., Gisbert, D., Flaquer, C., Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions, *Mammalian Biology* (2015), <http://dx.doi.org/10.1016/j.mambio.2015.03.008>

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Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions

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Abstract

Pest control through integrated pest management systems stands as a very convenient sustainable hazard-free alternative to pesticides, which are a growing global concern if overused. The ability of the soprano pipistrelle bat (*Pipistrellus pygmaeus*) to control the rice borer moth (*Chilo suppressalis*), which constitutes a major pest of rice around the world, was studied in the Ebre Delta, Northeastern Iberia. Evidence was found on the ability of this particular bat species to control borer infestations: a) the moth was consumed during at least the last two peaks of the moth activity, when most crop damage is done; b) the activity of bats significantly increased with moth abundance in the rice paddies; c) the pest levels have declined in the study area (Buda Island, Eastern Ebre Delta) after the deployment of bat boxes and their subsequent occupation by soprano pipistrelles. The value of the ecosystem service provided by bats was estimated at a minimum of 21€ per hectare, equivalent to the avoided pesticide expenditure alone. We suggest that this natural service can be enhanced by providing bat populations with artificial roosts in rice paddies were some key ecosystem features are present.

Keywords: rice paddies, *Pipistrellus pygmaeus*, *Chilo suppressalis*, ecosystem services, ecological functions, integrated pest management, biological control

Introduction

"Any genuine improvement in the human condition on this planet must be concerned with rice" (Heinrichs and Miller, 1991). In recent decades, both rice scientists and farmers have gained experience in the cultivation of rice and there has been a shift from a primarily unilateral approach to insect pest control, relying strongly on insecticides, to a multilateral approach involving a combination of control tactics (Heinrichs and Miller, 1991). Scientists throughout the world strive to develop and implement strategies to control rice pests more effectively and economically, to improve crop productivity and consequently the welfare of human populations. According to Food and Agriculture Organization of the United Nations (FAO) statistics, the global production of rice increased linearly from 216 million tons (mt) in 1961 to 722 mt in 2011 (FAO). New strategies to control insect pests include the use of one insecticide per one specific target species combined with biological methods (such as the use of parasitoids). However, the adoption of non-chemical approaches to pest control is not evenly distributed around the world and occurs mainly in those regions where legal constraints limit the number of approved chemical products (c. a. European Union). Over-use of pesticides is still an issue to be addressed (Normile, 2013; Peng et al., 2009), particularly in those regions in which the vast majority of the world's rice production is concentrated, and the use of pesticides keeps growing (FAO).

Fifty percent of the insecticides used in rice fields in Asia target lepidopteran insects (Heong et al., 1994). In 1991 it was estimated that an average global annual yield loss of 10 million tons was caused by just three moths: the striped rice borer (*Chilo suppressalis*), the yellow stem borer (*Scirpophaga incertulas*) and the leaf folder (*Cnaphalocrocis medinalis*) (Herdt, 1991). The striped rice borer is an Asian moth currently present in paddies worldwide. The adults lay their eggs on the stems and leaves of rice plants, and the larvae bore the stems to feed on the internal tissues, compromising both plant growth and productivity, sometimes fatally.

In Europe most bats are insectivorous. Bats may eat up to 80-100% of their body mass in insects on a nightly basis (Kurta et al., 1989), and during the last decade several authors have drawn attention to the important contribution that bats make to insect pest control (Agosta and Morton, 2003; Boyles et al., 2011; Cleveland et al., 2006; Ghanem and Voigt, 2012; Kunz et al., 2011; Lee and McCracken, 2005; Leelapaibul et al., 2005; McCracken et al., 2012; Whitaker, 1995). The referred contribution has been assessed by the presence of pests in the diet of wild bat populations or by taking a step further and accounting for the economic value of such an ecosystem service. Given the

natural complexity of ecological systems it is difficult to place a monetary value on the services they provided by bats, a fact that restricts how their importance is understood by the public (Fisher and Turner, 2008). Cleveland et al. (2006) estimated the economic contribution of bats to the cotton dominated agroecosystems of southern Texas, USA, to be \$12-\$173 per acre each year. By extrapolating these figures to the whole country, Boyles et al. (2011) valued bats' economic contribution to the USA's agroecosystems at between \$3.7 and \$53 billion/year. Even if the actual figures were lower, given the evidence gathered so far, the positive impact of bats on this aspect of the economy seems to be beyond dispute. This benefit can exceed the monetary value if the affected crop is a staple. For example, the pest control service provided by wrinkle-lipped bats' (*Tadarida plicata*) is responsible for securing the meals of 26,152 ($\pm 15,817$ SD) people each year in Thailand alone (Wanger et al., 2014). All the aforementioned authors have stressed the consequent importance of protecting bat populations if the ecological service they provide is to be preserved.

Although there is no general agreement on how to define ecosystem services (Wallace, 2008), such services are generally regarded as ecosystem outcomes (e. g. use of less pesticides) that contribute to human well-being (Fisher and Turner, 2008; Fisher et al., 2009; Nelson et al., 2009; Wallace, 2007). Understanding the mechanisms that link ecological systems to human well-being is a fundamental task when studying ecosystem services (Haines-Young and Potschin, 2009). From a functional perspective, the human benefit (whether monetary or not) is the final outcome of a cascade process resulting in a service (Rollett et al., 2008).

The soprano pipistrelle (*Pipistrellus pygmaeus*, Fig. 1) was separated taxonomically from the common pipistrelle bat (*Pipistrellus pipistrellus*) on 1997 (Barratt et al., 1997). Previous studies on diet contain mixed data on diet and distribution (e.g. Vaughan, 1997). Soprano pipistrelle is a common European bat species occurring from the British Isles through much of continental Europe (including the islands of Corsica and Sardinia) East to Western Asia Minor, the Caucasus and Siberia (Dietz et al., 2009). It is more abundant in lowland areas and is frequently associated with freshwater bodies (rivers, lakes, wetlands, etc.), being common in coastal wetlands, where most paddies in southern Europe occur. The few unambiguous studies on diet indicate that they feed mainly on small diptera, though they include a wide array of small-sized aerial insects in their diet and is regarded as an opportunistic species (Bartonicka et al., 2008b; Vaughan, 1997). Following our success in improving soprano pipistrelle populations in Northeastern in a mixed landscape with both wetlands and paddies (Flaquer et al., 2006), we investigated the ability of the species to control

striped rice borer populations. Having determined the potential of bats to control rice borer, we aimed to better understand under which environmental conditions soprano pipistrelles' populations could be enhanced to further promote their pest control ability. Since the experiment was conducted in a reduced area, we describe the conditions under which bat populations had been encouraged in the rice producing landscape of study, and emphasise the economic benefits they could provide. Although there is a long tradition of rice-fish culture aimed both at controlling pests and enhance food production (Xie et al., 2011), to our knowledge no other wild local vertebrate populations have been artificially manipulated for such a purpose and no previous research exists on enhancing bat populations as a pest biological control method in rice paddies.

Material and methods

Study area

We carried out the study within the Ebre Delta Natural Park ($0^{\circ} 50' E$, $40^{\circ} 42' N$, Figure 2), Catalonia, NE Iberia, which is one of the biggest deltas in Europe (320 km^2). Approximately 65 km^2 are wetlands, 240 km^2 are crops (203 km^2 of which are paddies) and 16 km^2 are urban areas that host around 5,000 inhabitants. Mean annual temperatures range between 17 and 18°C , mean annual precipitation between 500 - 550 mm, and mean annual evapotranspiration between 855 - 997 mm, in what is essentially a semi-arid Mediterranean climate, with a pronounced summerdrought.

Prior to this study, in 1999, 69 small wooden bat boxes, either with single or double compartment (Flaquer et al., 2006), were erected in Buda Island Natural Reserve (12.1 km^2 , Figure 2), a deforested area lacking suitable vertical structures for the bats to roost in (Flaquer et al., 2005). Bat boxes were rapidly occupied (243 individuals were counted one year after boxes were deployed) by an increasing number of individuals, reaching a maximum of approximately 3,500 individuals in 2008 (Flaquer et al., 2006).

Pest control systems

Following Cleveland et al. (2006) we can estimate the value of the service provided by bats in two ways: 1/ assessing the economic expenses that were reduced/avoided due to bat predatory activity, and 2/ assessing the value of the crops that remained undamaged. The latter is harder to assess since some of the measurements required are difficult to gather in the wild (e.g. the total number of bats hunting per unit area of rice fields in a period of time). Thus, the conservative figure that is most easily calculated from the available local data is the expenditure on chemical treatments that was avoided in the 2.9 km^2 of paddies in Buda Island crops between 2006 and 2012.

Pest control procedures in the region are done by farmers in coordination with the Catalan Government that have created the Plant and Crop Protection Association in the Ebre Delta. These procedures consist of a permanent network of trapping devices lured with chemical attractants targeted at rice borer males. These are deployed at a rate of 4.5 traps per hectare all over the 203 km² of paddies, totalling approximately 91,300 traps at an estimated annual expenditure of 275,000 €. In addition to this non-hazardous biological method, a moth surveillance programme measuring the density of affected rice stems is done throughout the area during both the second and the third peak of the rice borer's activity. Aerial spraying with tebufenocide, at a cost of 21€/hectare, is applied in areas where the presence of rice borer eggs or caterpillars exceeds 0.85 rice stems/m² during the second peak of borer activity, or 2.15 rice stems/m² during the third and final peak. The effectiveness of the described treatments, measured as the reduction of striped rice borer moth density, is estimated to average around 70% for trapping and 35% for aerial spraying (ADV – Crop Defence Association).

Rice borer consumption by bats

Chilo suppressalis species – specific primers for 28S-D2 and mitochondrial COI genes have been designed in order to determine its presence in the soprano pipistrelles fecal DNA.

DNA extraction from rice borer, amplification and sequencing

The 5' region of *C. suppressalis* cytochrome oxidase I (COI) gene was recovered from GeneBank whilst the COI 3' region and the 28S-D2 sequences were not available. To obtain these sequences amplifications and sequencing of 28S-D2 and the 3' region of the COI gene were performed on rice borer DNA by using two different set of universal primers, ND2F/ND2Rev (Campbell et al., 1993) and C1-J- 2183/TL2-N-3014) (Simon et al., 1994) respectively.

DNA extraction from the borer was carried out by homogenizing the head, one forewing and one leg with a plastic pestle, and treating them with Chelex resin/proteinaseK (Vickerman et al., 2004).

The PCR cycling program for 28S-D2 primers pair was: 3min at 94°C, followed by 35 cycles of 45s at 94 °C, 1min at 52 °C, 2min at 72°C, and a final extension of 7min at 72°C. For the COI gene the PCR cycles were the same as described for 28S-D2 except that the annealing temperature was lowered to 48°C. All the amplifications were carried out in a 40µl reactions using 4µl of DNA template, 1x buffer (Promega), 0.2 mM of each dNTP, 10 pmol of each primer and 0.6 units of GoTaq

DNA polymerase (Promega), and checked on 1% agarose gel stained with ethidium bromide.

The amplicons were directly sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready ReactionKit (PE Applied Biosystems), on the ABI PRISM 310 DNA Sequencer. Primers specific for *C. suppressalis* 28S and COI shorter internal portions were designed (see supplementary material for PCR primers, length of amplicons and amplifications conditions) with the software Primer-blast at NCBI (Rozen and Skaletsky, 2000). It uses Primer3 to design PCR primers and then submits them to BLAST search against user-selected database. In this study the database was made for all Lepidoptera other than *C. suppressalis* known sequences of the same regions. The results are then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template.

Bat droppings collection

During 2008 and 2009 we analyzed 80 bat droppings (30 + 50 respectively) to determine whether the pest under study was being consumed by the soprano pipistrelles. All the droppings were

collected from 50 bat boxes in Buda Island Natural Reserve. In 2008, all samples were collected during June, thus corresponding to the second peak of the Rice borer, while in 2009 all droppings were collected in August, during the third and highest peak using the same method. In all cases the droppings were preserved in alcohol 70%.

DNA extraction from bat droppings

Droppings were air dried on filter paper to remove as much ethanol as possible, then placed into eppendorf tubes containing 150 µl of extraction buffer (10 mM tris HCl pH 8.0, 1 mM EDTA, 1% Nonidet P-40, 200 µg/ml Proteinase K) vortexed briefly, incubated overnight at 55°C, and 2 h at - 20°C. Samples were then subjected to a second round of lysis by adding 50 µl of extraction buffer, and incubation at 55°C for 2h. Proteinase K was inactivated heating the sample 10 minutes, and DNA recovered after centrifugation of 10 minutes at 14.000 rpm. The pellet containing the dropping debris was utilized for microscopy analysis. Each sample was split in two, and one of the portions was saved for later analysis. As positive control a small fragment of an antenna and a tarsum of the borer (size of fragments similar to that retrieved in bats' droppings) were added to two different faeces samples prior to perform DNA extraction (to be sure that there are not compounds inhibiting the PCR in the faeces). As negative control,

DNA was extracted with the same protocol described above, from fragments retrieved in bat droppings and identified by microscopy analysis as "not Lepidoptera" specimens; they were two legs, a head, and head with antenna.

Morphological analysis of insects fragments found in bat droppings

Before running morphological analysis, two slides (one male and one female) of the borer were prepared following Noyes (1982) (mounting processes used for parasitoid insects) for identifying some key morphological characters that could help in identifying putative fragments in the bat droppings. Morphological analysis of the droppings was performed on all samples after DNA extraction, by crumbling gently the droppings in absolute ethanol and by examining at stereo-binocular (30x) the single fragments to look for those that could belong to *C. supressalis*. Putative fragments were slide mounted and compared with homolog parts on the slides (male and female) of *C. supressalis*.

Activity patterns

In southern European paddies, three generations of rice borer occur in a year, resulting in three moth peaks during the rice crop growing season from May to September (Figure 4). To ascertain the flight activity patterns of the striped rice borer populations, including both males and females, we distributed a total of eight light traps throughout the Ebre Delta, and monitored each light trap continuously from mid May to late September during four years (2007-2010). We assessed moth activity by counting the individuals caught in traps every 2-3 days. In order to ensure that data on rice borer moth activity was comparable to bat activity data, we pooled the counts in ten day intervals.

We quantified total bat activity as passes per minute by means of acoustic surveys and aimed to count *in situ* the number of bat passes around 53kHz (the fundamental frequency most used by soprano pipistrelles in the area) using the heterodyne system. We used Pettersson D240x bat detectors (Pettersson Elektronik AB, Sweden) with heterodyne and time expansion (x10) systems, and a digital recorder (Edirol R9) to randomly record time expanded samples of the echolocation and social calls to verify the field identifications in the laboratory (BatSound Software, Pettersson Elektronik). All the field work was undertaken by the same researcher to prevent differences in the identification skills of individuals to interfere in the results (Limpens, 2004). We collected data at randomly selected points in three different years; survey effort varied year to year according to the budget available. Each point (totally 87) has been surveyed only one year but in different occasions in order to have correlated data with moth activity from the same year. During 2007 we counted bat passes at 40 points (32

in rice paddies and 8 in reed beds *Phragmites spp.*, as a control). Each point survey lasted five minutes and was located more than 100m apart from the nearest station to avoid pseudoreplication. In order to observe differences in bat foraging activity during the striped rice borer flight period, we surveyed each point eight times from early July to mid September every 10 days. During each survey we began the sampling process from a different point to avoid time biases, and all stations were surveyed on the same night within a three hour period. The same approach was performed in 2009 with 20 stations (17 in rice and 3 in reed beds) and four temporal replications (from late August to mid September), centring the sampling on the period when the third and highest moth peak occurs. Finally, in 2010 the acoustic survey consisted of three minute samples at 27 points (22 in rice and 5 in reed beds) with six temporal replications, from August to mid September. A total of 87 acoustic stations (71 in rice) produced 2,486 minutes of recordings (82% in rice paddies). All stations were sampled under similar climatic conditions, avoiding sampling in windy and/or rainy days.

Rice borer activity patterns between the four years were analysed by means of simple correlations, thus making all pair-wise comparisons for the average number of moths counted in the eight light-traps per sampling session (a total of 18 sessions from May to September).

We used differences between the mean activity of bats in rice paddies and in control areas (reed beds) to reveal the relationship between bat and rice borer moth activity. The former was considered to be the dependent variable that would respond to the rice borer population levels.

To examine bat activity in relation to moth activity while accounting for the effects of habitat, sampling location or time of survey, we used Generalized Linear Mixed Models (GLMMs, Bolker et al. (2009)). According to Gotelli and Ellison (2004) we assumed bat activity, which measures the number of occurrences of bat passes in a fixed interval of time, to be Poisson distributed. We introduced moth counts and month of sampling in the model as independent continuous variables. The model also accounted for the effects of habitat as an independent (fixed) factor. Since some sampling locations differed among years we introduced the former as a random effect nested within the sampling year.

Prey-predator associations alone constitute unreliable evidence of the ability of a particular predator to control its prey populations, since p.e. the predator may only feed on certain prey when the latter is very abundant, and thus the predatory pressure may not have any effect on its populations (Arditi and Dacorogna, 1988). With the aim of examining at which level of rice borer pest activity the bats were significantly attracted

to the rice paddies, we arranged the data to meet the needs of an analysis of variance by generating two datasets: 1/ grouping moth activity into four categories using the quartiles of its density distribution, and 2/ grouping the same variable into two binary categories (Rice borer moths present or absent). Given the non-normality of the distribution of the dependent variable ($p<0.01$ in the Kolmogorov-Smirnov test of normality in both raw and log-transformed data), we used a non-parametric approach to test for differences in bat activity between the groups described. We used the Kruskal-Wallis by Ranks Test in the first case (four categories of the predictor available), and the Mann-Whitney U Test for the second one. We performed *post-hoc* multiple comparisons of mean ranks when results were significant to test for differences between all pairs of groups. Statistica 7.0 (StatSoft Inc., Tulsa, USA) and R 3.0.1 (R Development Core Team, 2014) were used to compute the analysis.

Habitat quantification within the study area

. We used land cover maps produced by the Catalan Government (CREAF, 2009) to quantify the key habitats present within soprano pipistrelle territories around the occupied bat boxes in Buda Island. We reclassified land use into ecologically meaningful categories based on the habitat selection studies available for this species (Bartonicka et al., 2008a; Boughey et al., 2011; Davidson-Watts et al., 2006), maintaining those categories relevant to the present research: a) riparian forests; b) marshes and wetlands; c) river (water surface and unforested riversides); d) rice paddies; e) herbaceous vegetation; f) urban and suburban; and g) others. We calculated a 2500 metres buffer (19.63 km²) around the area with the occupied bat boxes existing on Buda Island, as an approximation of the reported foraging areas for breeding colonies (Davidson-Watts et al., 2006; Nicholls and Racey, 2006). To test for differences in the available habitat between Buda Island and the rest of the Delta we estimated habitat composition around 69 points that were randomly distributed across the whole Ebre Delta and compared the mean coverage of each habitat category in the random sample with its mean coverage in the buffer around the 69 bat boxes with a Monte Carlo randomized analysis (Gotelli and Ellison, 2004). Geographical data extraction, analysis and representation was run under R 3.0.1 (R Development Core Team, 2014).

Service valuation and Minimum Safe Unit

We estimated the value of the service provided by bats using the avoided-cost approach, which accounts for the expenditure prevented by a service, following the method of Cleveland et al. (2006). We included only those figures that were readily measurable (cost of the avoided treatments) and avoided accounting for less

measurable economical benefits (undamaged crops or avoided social cost of pesticides) that would increase the uncertainty of the result and the number of assumptions to be made.

Implementing the concept of Minimum Safe Unit (Luck et al., 2009), defined as the minimum amount of an element or set of elements of the ecosystem necessary to ensure a particular function that brings about a desired service, we estimated the number of soprano pipistrelles per hectare needed to maintain the striped rice borer populations below two different thresholds that are used locally by farmers to trigger the pest treatments: no treatment needed (<3,500 moths/ha) and aerial treatment needed (>8,500 moths/ha).

Results

Rice borer predation

During summer inspections of the roosts, bats were frequently observed hunting rice borer moths. In 2008, six out of thirty bat droppings (20%) were positive for the presence of striped rice borer during the second peak of borer activity (mid July). In 2009, the screening was conducted with samples from the third peak (last week of August to mid September) and resulted in 25 out of 50 positive results (50%). Temporal distribution of the average number of moths trapped per every ten days in the eight light traps were significantly correlated among years (2007-2010, mean correlation coefficients for six pair-wise comparisons: $r = 0.76$, range 0.67-0.86, all $p < 0.05$; $n = 18$ sampling periods), suggesting that moth dynamics was similar among the study years, showing lows and peaks at similar dates.

Bat activity was strongly associated to foraging events since bat passes were highly correlated with hunting buzzes in the three study years (year 2007: $r = 0.80$, $p < 0.0001$, $n = 320$; year 2009: $r = 0.90$, $p < 0.0001$, $n = 80$; year 2010: $r = 0.93$, $p < 0.0001$, $n = 162$). Between July and September (data pooled from the three years of sampling) the bat passes per minute in rice paddies averaged $2.9 \text{ SD} \pm 3.86$ (range 0 to 22, $n = 456$). In the control areas, mean bat passes per minute were $1.18 \text{ SD} \pm 2.38$ (range 0 to 11, $n=106$).

Bats significantly increased their activity when rice borer density increased (table 1, GLMM: $|z| = 8.243$, $p < 0.0001$), and showed a lower activity within the reed beds (table 1, GLMM: $|z| = 4.230$, $p < 0.0001$). Bat activity was significantly higher in the rice paddies than in the reed bed control plots provided the rice borer moths were present

at any level ($U = 8450.0$; $p < 0.001$, $df = 541$). Conversely no such difference was found when moths were absent from light trap catches ($U = 35.0$, $p > 0.5$, $df = 18$; Figure 3). Bat activity did not vary significantly among the four quartiles of moth density in the reed beds ($K_{3, 80} = 6.848$, $p > 0.05$), however there was a significant increase in bat activity with rising levels of moth activity in the rice paddies ($H_{3, 463} = 36.433$, $p < 0.0001$). Bats increased significantly their activity in the rice fields when a threshold of two moths per light trap per day was reached ($z < 2.98$, $p < 0.05$) as revealed by the comparisons between groups (Figure 3). Figure 4 depicts the idealised yearly activity cycle of the rice borer and the threshold at which bats significantly increase their foraging activity in the rice fields: even the lowest first generation of the moth attracts bats.

Interestingly, the experiment of deploying bat boxes on Buda Island reported an important increase of the bat population in the area over a ten year period ($r = 0.95$, $p < 0.001$, $n = 10$, Figure 5). Bats started occupying boxes in the year 2000 (243 individuals were counted one year after bat box installation in 1999), and reached a maximum count of 3,500 ind. in 2007 and 2008 (the last years with a census). During the same period a significant decrease in the number of rice stems/m² infested by the rice borer was detected in the area ($r = -0.73$, $p < 0.01$, $n = 10$, Figure 5). The bat density in the boxes was negatively correlated to rice damage over a ten year period ($r = -0.64$, $p < 0.05$, $n = 10$).

Habitat composition around bat boxes

Wetlands, rivers, riparian forests and herbaceous vegetation were significantly more available inside the 2.5 km buffer surrounding the Buda Island breeding colonies than around the random points that were scattered across the whole Ebre Delta ($|z| > 3.50$; $p < 0.001$), whereas there was no such difference regarding the coverage of cultivated land (rice paddies), urban structures or other land cover ($|z| > 3.50$; $p > 0.09$). Around the occupied bat box stations ($n=69$) the mean coverage of riparian forest was around 0.5%, rivers accounted for between 6-7% and urban structures ranged between 6-10% of the available area. Rice paddies were the dominant habitat, covering between 50-64% of the land, followed by marshes from 11% to 24%.

Service valuation

We estimated the cost of every chemical treatment is 21€ per hectare, i.e. roughly 6.000€ in less than 3km². According to our estimates (see Figure 6) between 9 and 16 bats per hectare are needed to reduce the number of stems affected by the striped rice borer during their second generation to less than 0.85 affected stems/m² (aerial

treatment threshold). Between 42 and 67 bats per hectare would be needed to further lower the moth density below any treatment triggering threshold (0.35 affected stems/ m^2). Assuming that the population of bats in Buda Island is represented by the colonized bat boxes (around 3,500 bats), we can consider that during the study period there were around 12 hunting bats per hectare in this area.

Discussion

Any biological pest control method capable of diminishing or even preventing the use of chemical treatments is to be encouraged where there is a concern for environmental and human health. Our results suggest that soprano pipistrelle bats provide natural control of a pest, the striped rice borer moth in the Ebre Delta. Evidence of this control is based on two related results: 1) soprano pipistrelle bats preyed upon striped rice borer moth, even during the second (lower) peak of borer activity; 2) activity of bats tracked the pest moth abundance in rice paddies. Besides, since 2006 borer density has been below the threshold for aerial spraying (*i.e.* below 0.85 stems hosting either larvae or eggs per square meter during the moths' second generation). A significant decline in the abundance of infested plants in the Buda Island could be partially explained by the presence of a bat population (in bat boxes) that is large enough to reduce rice borer populations, but this topic should be studied in depth.

Though larvae infestation surveys were conducted systematically over the whole Ebre Delta during the period covered in this study that trend was not reported anywhere else in the Ebre Delta (ADV –Crop Defense Association- data). The results obtained from this study provide further evidence that soprano pipistrelles habitually prey on the pest and move into the paddies even when few moths are available (Figure 3), responding to striped rice borer emergence by foraging more on the rice paddies when the aerial phase of the pest is present. Bats increase their activity on the rice fields well before the third and highest peak. This observed opportunistic feeding behaviour seems typical of this species (Bartonicka et al., 2008b).

From the evidence gathered, the ability of soprano pipistrelles to control rice borer population levels stands as the most parsimonious explanation for the decrease of the pest in the Buda Island rice paddies, where there are no less than 3,500 bats, corresponding to a density of 12 bats per hectare of paddies within the range of influence of bat boxes (Flaquer et al., 2006). So, we have tried to approximate the density of bats per hectare required to keep the stripped rice borer population below

the threshold for aerial treatment in the study area (Fig. 4). When properly located (e.g. selecting the appropriate support and exposure, Flaquer et al. (2014), bat boxes are readily accepted as roosting places by bats in the area (Flaquer et al. 2006), and can be made at minimal cost by schoolchildren on environmental awareness programmes. Even when ready-made boxes are bought by farmers, each wooden roost costs around 25€ and lasts more than 10 years without any maintenance. In contrast, the cost of pheromone traps can be around 15€/ha per year (3€/trap x 5 traps/ha), while spraying one hectare once a year represents about 21€/ha per year. In practice, annual costs of chemical spraying are often higher because a second treatment is usually made if the third moth peak is severe. Therefore, the estimates on both chemical treatments are 6-8 fold higher than the installation of bat boxes accounting for a minimum of 12 bats per box.

In other areas of the world with dense human populations bat roosts are known to contribute to the reduction of pest damage on crops, e.g. in the North American continent (Boyles et al., 2011; Cleveland et al., 2006; McCracken et al., 2012) or Asia (Wanger et al., 2014). In both these areas the molossids (family *Molossidae*) involved in pest control gathered in huge colonies of up to millions of individuals, and foraged at a large regional scale. Molossids are fast flying species compared to many vespertilionids, including the pipistrelles, which fly at significantly lower speeds (Norberg and Rayner, 1987). Consequently the potential area for pest control by soprano pipistrelle colonies is significantly smaller, a fact that brings about new management challenges. Finding out which habitat features best encourage the presence of soprano pipistrelles would allow local management strategies to establish populations in target agroecosystems, to deliver the pest control ecosystem service.

Habitat availability around selected bat boxes of Buda Island is consistent with the preferences for the species described in other studies from central and eastern Europe (Bartonicka et al., 2008a; Boughey et al., 2011; Davidson-Watts et al., 2006), showing a reliance on flooded habitats (e.g. marshes and rivers) and broadleaved forests (e.g. riparian woodlands). The rapid colonisation of the bat boxes that were installed in the area in 1999 and the subsequent growth of the local bat population suggests that the limiting factor for this bat population was the existence of suitable roosts (Flaquer et al., 2006), given the lack of vertical structures that could provide shelter. The soprano pipistrelle is one of the most common bat species in southern Europe (Dietz et al., 2009), and can reach very high densities locally (Flaquer et al., 2006).

According to Wallace (2007) we should focus adequately managing ecosystem features to ensure the delivery of a service that will eventually improve our well-being. Remarkably, a simple management action, such as erecting bat boxes, can catalyse the performance of a desirable ecosystem service. Other examples of manipulating paddy habitats for enhancing ecosystem services can be found in Vietnam, where simple low-cost actions such as planting flowers and vegetables on the banks of the paddies have enhanced the abundance of the bees and wasps that parasitise and help control some of the insect pests (Normile, 2013). Laboratory studies of Indian meal moth (*Plodia interpunctella*), a pest moth belonging to the same super-family as the rice borer *Piraloidea* (Regier et al., 2012), have found that in the presence of ultrasound mating behaviour, spermatophore transfer, egg production and larval weight are significantly reduced (Huang and Subramanyam, 2004; Huang et al., 2003). Like the Indian Mealworm, rice borers possess tympanal organs and should therefore be capable of hearing foraging bats. If a similar response to ultrasound were observed in striped rice borer, it is possible that the presence of foraging pipistrelles in the fields could also stimulate lower reproductive rates in the moth, making a further contribution to its biological control. Though further research is necessary to test this theory, we hypothesise that several bat boxes distributed along the rice paddies could be more effective than huge artificial bat roosts.

In conclusion we have evidence that the soprano pipistrelle habitually and opportunistically preys on the striped rice borer moth in rice paddies and we have observed negative pest trends in an area with thousands of bats roosting in bat boxes (>12 bats per hectare).

Acknowledgements

Bats were caught under permit from the Ministry of the Environment and Housing of the Catalan Government (Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural). Our most sincere gratitude is for Danilo Russo, who gave us the opportunity to contribute to this special issue. We are very grateful to Karen Hayson and to the anonymous referees for their comments on the manuscript. We also acknowledge statistical advice from Prof. Mario Díaz (CSIC). This work was supported by the Catalan Government (Generalitat de Catalunya). We would also like to thank the Ebre Natural Park staff - above all, Francesc Vidal, Toni Curcó, Miquel Àngel Franch,

Vicente Fouces, Xavi Porres and Ramon Bartomeu. We have to thank Antoni Arrizabalaga Blanch (Director of the Granollers Museum of Natural Sciences) for the years spent in training us. This project was partially funded by the Ministerio de Economía y Competitividad of the Spanish Government (Ayudas para la Realización de Proyectos de Investigación, Subprograma Investigación Fundamental no Orientada 2012 . ref. CGL2012-38610).

References

- Agosta, S.J., Morton, D., 2003. Diet of the big brown bat, *Eptesicus fuscus*, from Pennsylvania and western Maryland. *Northeast Nat* 10, 89-104.
- Arditi, R., Dacorogna, B., 1988. Optimal Foraging on Arbitrary Food Distributions and the Definition of Habitat Patches. *Am Nat* 131, 837-846.
- Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P., Wayne, R.K., 1997. DNA answers the call of pipistrelle bat species. *Nature* 387, 138-139.
- Bartonicka, T., Bielik, A., Rehak, Z., 2008a. Roost switching and activity patterns in the soprano pipistrelle, *Pipistrellus pygmaeus*, during lactation. *Ann Zool Fenn* 45, 503-512.
- Bartonicka, T., Rehak, Z., Andreas, M., 2008b. Diet composition and foraging activity of *Pipistrellus pygmaeus* in a floodplain forest. *Biologia* 63, 266-272.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24, 127-135.
- Boughen, K.L., Lake, I.R., Haysom, K.A., Dolman, P.M., 2011. Effects of landscape-scale broadleaved woodland configuration and extent on roost location for six bat species across the UK. *Biol. Conserv.* 144, 2300-2310.

Boyles, J.G., Cryan, P.M., McCracken, G.F., Kunz, T.H., 2011. Economic Importance of Bats in Agriculture. *Science* 332, 41-42.

Campbell, B.C., Steffen-Campbell, J.D., Werren, J.H., 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol. Biol.* 2, 225–237.

Cleveland, C.J., Betke, M., Federico, P., Frank, J.D., Hallam, T.G., Horn, J., López, J.D., McCracken, G.F., Medellín, R.A., Moreno-Valdez, A., Sansone, C.G., Westbrook, J.K., Kunz, T.H., 2006. Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. *Front Ecol Environ* 4, 238-243.

CREAF, 2009. Mapa de Cobertes del Sòl de Catalunya, 4a edició. Generalitat de Catalunya.

Davidson-Watts, I., Walls, S., Jones, G., 2006. Differential habitat selection by *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. *Biol. Conserv.* 133, 118-127.

Dietz, C.D., Helversen, O.v., Nill, D., 2009. Bats of Britain, Europe and Northwest Africa. A. & C. Black, London.

FAO (Food and Agriculture Organization). <http://faostat.fao.org>, last accessed 12 March 2013

Fisher, B., Turner, R.K., 2008. Ecosystem services: Classification for valuation. *Biol. Conserv.* 141, 1167-1169.

Fisher, B., Turner, R.K., Morling, P., 2009. Defining and classifying ecosystem services for decision making. *Ecol Econ* 68, 643-653.

Flaquer, C., Puig-Montserrat, X., López-Baucells, A., Torre, I., Freixas, L., Mas, M., Porres, X., Arrizabalaga i Blanch, A., 2014. Could overheating turn bat boxes into death traps? *Barbastella* 7, 46-53.

Flaquer, C., Ruiz-Jarillo, R., Torre, I., Arrizabalaga, A., 2005. First resident population of *Pipistrellus nathusii* (Keyserling and Blasius, 1839) in the Iberian Peninsula. *Acta Chiropt* 7, 183-188.

Flaquer, C., Torre, I., Ruiz-Jarillo, R., 2006. The value of bat-boxes in the conservation of *Pipistrellus pygmaeus* in wetland rice paddies. *Biol. Conserv.* 128, 223-230.

Ghanem, S.J., Voigt, C.C., 2012. Increasing Awareness of Ecosystem Services Provided by Bats. *Advances in the Study of Behavior* 44, 279-302.

Gotelli, N.J., Ellison, A.M., 2004. A primer of ecological statistics. Sinauer Associates, Inc. Publishers, Sunderland, Mass.

Haines-Young, R., Potschin, M.B., 2009. Methodologies for defining and assessing ecosystem services. Final report, JNCC, Project Code C08-0170-0062, p. 69.

Heinrichs, E.A., Miller, T.A., 1991. Rice insects: management strategies. Springer, New York, N.Y.

Heong, K.L., Escalada, M.M., Mai, V., 1994. An Analysis of Insecticide Use in Rice - Case-Studies in the Philippines and Vietnam. *Int J Pest Manage* 40, 173-178.

Herdt, R.W., 1991. Perspectives on Agricultural Biotechnology Research for Small Countries. *J Agr Econ* 42, 298-308.

Huang, F.N., Subramanyam, B., 2004. Behavioral and reproductive effects of ultrasound on the Indian meal moth, *Plodia interpunctella*. *Entomol Exp Appl* 113, 157-164.

Huang, F.N., Subramanyam, B., Taylor, R., 2003. Ultrasound affects spermatophore transfer, larval numbers, and larval weight of *Plodia interpunctella* (Hubner) (Lepidoptera : Pyralidae). *J Stored Prod Res* 39, 413-422.

Kunz, T.H., de Torrez, E.B., Bauer, D., Lobova, T., Fleming, T.H., 2011. Ecosystem services provided by bats. NY Acad Sci 1223, 1-38.

Kurta, A., Bell, G.P., Nagy, K.A., Kunz, T.H., 1989. Energetics of Pregnancy and Lactation in Free-Ranging Little Brown Bats (*Myotis lucifugus*). Physiol Zool 62, 804-818.

Lee, Y.F., McCracken, G.F., 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86, 67-76.

Leelapaibul, W., Bumrungsri, S., Pattanawiboon, A., 2005. Diet of wrinkle-lipped free-tailed bat (*Tadarida plicata* Buchannan, 1800) in central Thailand: insectivorous bats potentially act as biological pest control agents. Acta Chiropt 7, 111-119.

Limpens, H., 2004. Field identification: using bat detectors to identify species, Bat echolocation research: tools, techniques and analysis. Bat Conservation International Austin.

Luck, G.W., Harrington, R., Harrison, P.A., Kremen, C., Berry, P.M., Bugter, R., Dawson, T.P., de Bello, F., Diaz, S., Feld, C.K., Haslett, J.R., Hering, D., Kontogianni, A., Lavorel, S., Rounsevell, M., Samways, M.J., Sandin, L., Settele, J., Sykes, M.T., van den Hove, S., Vandewalle, M., Zobel, M., 2009. Quantifying the Contribution of Organisms to the Provision of Ecosystem Services. Bioscience 59, 223-235.

McCracken, G.F., Westbrook, J.K., Brown, V.A., Eldridge, M., Federico, P., Kunz, T.H., 2012. Bats Track and Exploit Changes in Insect Pest Populations. PLoS ONE 7.

Nelson, E., Mendoza, G., Regetz, J., Polasky, S., Tallis, H., Cameron, D.R., Chan, K.M.A., Daily, G.C., Goldstein, J., Kareiva, P.M., Lonsdorf, E., Naidoo, R., Ricketts, T.H., Shaw, M.R., 2009. Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. Front Ecol Environ 7, 4-11.

Nicholls, B., Racey, P.A., 2006. Contrasting home-range size and spatial partitioning in cryptic and sympatric pipistrelle bats. *Behav. Ecol. Sociobiol.* 61, 131-142.

Norberg, U.M., Rayner, J.M.V., 1987. Ecological morphology and flight in bats (Mammalia, Chiroptera) - wing adaptations, flight performance, foraging strategy and echolocation. *Philos T Roy Soc B* 316, 337-419.

Normile, D., 2013. Vietnam turns back a 'tsunami of pesticides'. *Science* 341, 737-738.

Noyes, J.S., 1982. Collecting and Preserving Chalcid Wasps (Hymenoptera, Chalcidoidea). *J Nat Hist* 16, 315-334.

Peng, S.B., Tang, Q.Y., Zou, Y.B., 2009. Current Status and Challenges of Rice Production in China. *Plant Production Science* 12, 3-8.

R Development Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Regier, J.C., Mitter, C., Solis, M.A., Hayden, J.E., Landry, B., Nuss, M., Simonsen, T.J., Yen, S.H., Zwick, A., Cummings, M.P., 2012. A molecular phylogeny for the pyraloid moths (Lepidoptera: Pyraloidea) and its implications for higher-level classification. *Syst Entomol* 37, 635-656.

Rollett, A., Haines-Young, R., Potschin, M.B., Kumar, P., 2008. Delivering environmental services through agri-environment programmes: a scoping study. Land use Policy Group, Contract No. EO0300011 - FC 73-03-03-54

Rozen, S., Skaletsky, H.J., 2000. Methods in Molecular Biology, in: Misener, S., Krawetz, S.A. (Eds.), Bioinformatics methods and protocols. Humana, Totowa, N.J., pp. 365-386.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene-Sequences and a Compilation of Conserved Polymerase Chain-Reaction Primers. *Ann Entomol Soc Am* 87, 651-701.

Vaughan, N., 1997. The diets of British bats (Chiroptera). Mammal Rev. 27, 77-94.

Vickerman, D.B., Hoddle, M.S., Triapitsyn, S., Stouthamer, R., 2004. Species identity of geographically distinct populations of the glassy-winged sharpshooter parasitoid *Gonatocerus ashmeadi*: morphology, DNA sequences, and reproductive compatibility. Biol Control 31, 338-345.

Wallace, K., 2008. Ecosystem services: Multiple classifications or confusion? Biol. Conserv. 141, 353-354.

Wallace, K.J., 2007. Classification of ecosystem services: Problems and solutions. Biol. Conserv. 139, 235-246.

Wanger, T.C., Darras, K., Bumrungsri, S., Tscharntke, T., Klein, A.M., 2014. Bat pest control contributes to food security in Thailand. Biol. Conserv. 171, 220-223.

Whitaker, J.O., 1995. Food of the Big Brown Bat *Eptesicus-Fuscus* from Maternity Colonies in Indiana and Illinois. Am Midl Nat 134, 346-360.

Xie, J., Hu, L.L., Tang, J.J., Wu, X., Li, N.N., Yuan, Y.G., Yang, H.S., Zhang, J.E., Luo, S.M., Chen, X., 2011. Ecological mechanisms underlying the sustainability of the agricultural heritage rice-fish coculture system. P Natl Acad Sci USA 108, E1381-E1387.

Table 1. GLMM Poisson regression results for the relationship between bat activity and: density of the moth *Chilo suppressalis*, habitat, and month of sampling (N = 543).

Effect	GLMM Estimate	Standard error	Z value	P value
Intercept	0.900	0.354	2.544	0.011
Density of rice borer	0.011	0.001	8.243	<0.0001
Habitat - reed	-0.942	0.222	-4.230	<0.0001
Month	-0.13511	0.042	0.048	0.962
Random effects				
Sampling location/year estimated variance \pm SD = 0.28 \pm 0.53				
Year estimated variance \pm SD = 0.08 \pm 0.29				



Figure 1.- A soprano pipistrelle (*Pipistrellus pygmaeus*) hunting a striped rice borer (*Chilo suppressalis*), in the Ebre Delta. Photographic stroboscopic effect (series of short or instantaneous samples); courtesy of Oriol Massana.

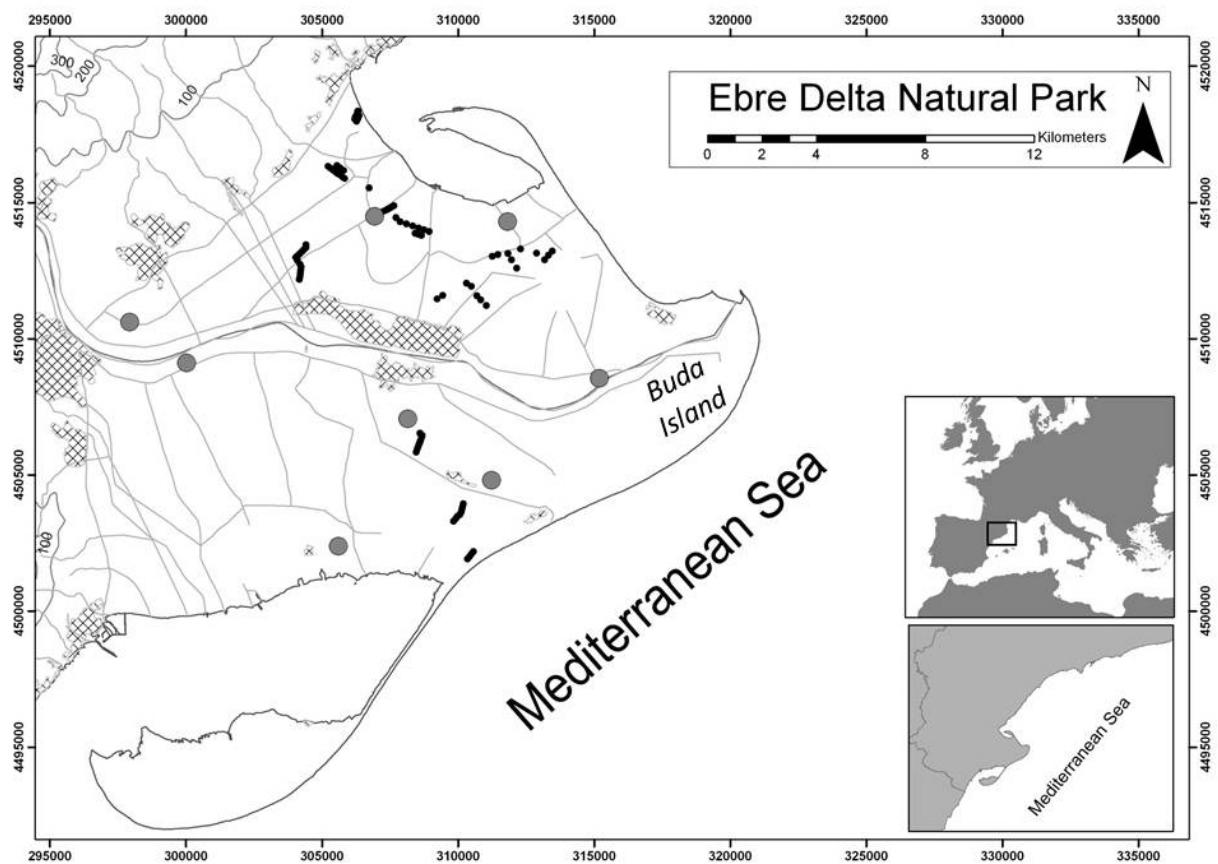


Figure 2. Situation of the study area and the sampling stations: Small black dots indicate the 87 independent bat detector stations (3-5 min duration) performed from July to September in 2007, 2009, and 2010, and large grey dots represent the location of the striped rice borer light-traps monitored from May to September 2007-2010. Shaded areas represent urban habitats.

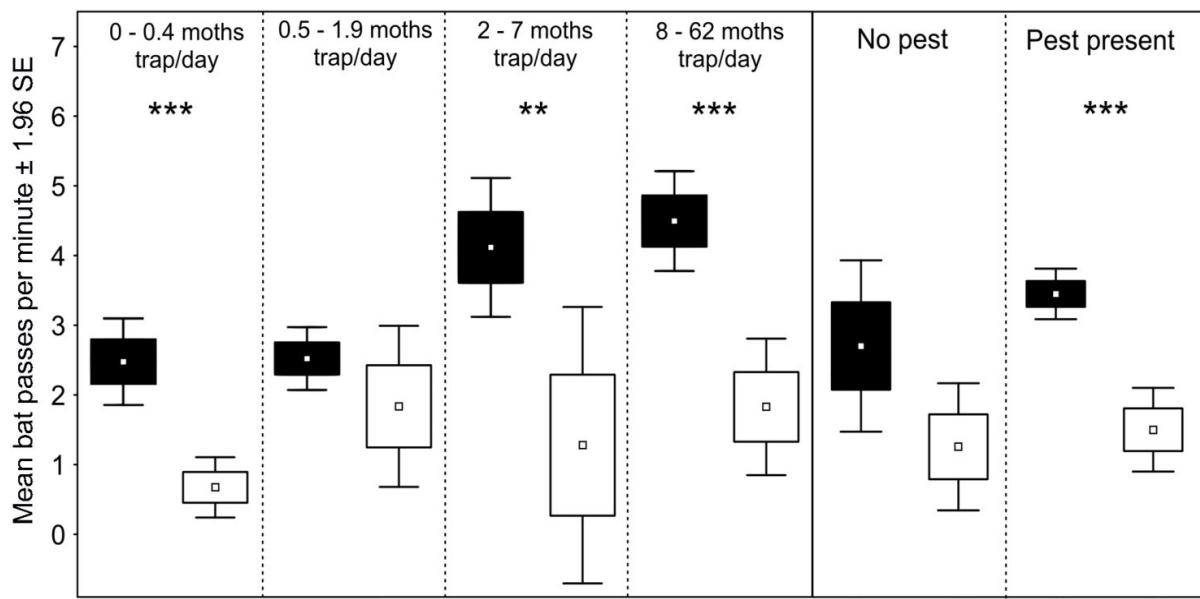


Figure 3.- Relationship between bats and striped rice borer activity. Black boxes represent bat passes (\pm SD and 95% confidence interval) in rice paddies and white boxes bat passes in reed-beds considered as a control habitat (***: $p < 0.001$; **. $p < 0.01$). Rice borer activity was grouped according to the four quartiles of its abundance counted at the light-traps.

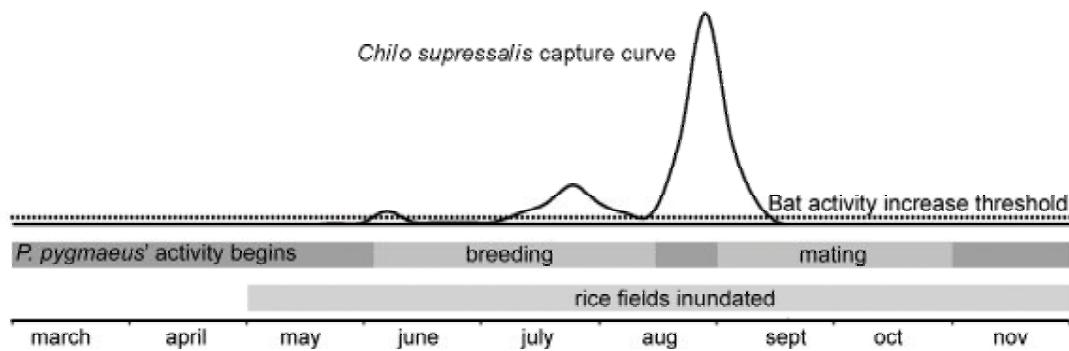


Figure 4.- Idealised timing of the activity cycle of the striped rice borer moth (*Chilo suppressalis*) according to the ADV Delta de l'Ebre - Ebre Delta Crop Defence Association (2005 to 2013) and activity cycle of the soprano pipistrelle (*Pipistrellus pygmaeus*). The dotted line indicates the 0.4 moths per trap threshold at which a significant difference between bat activity on controls and paddies appears (as shown in Figure 3). The period during which the paddies are flooded is also shown.

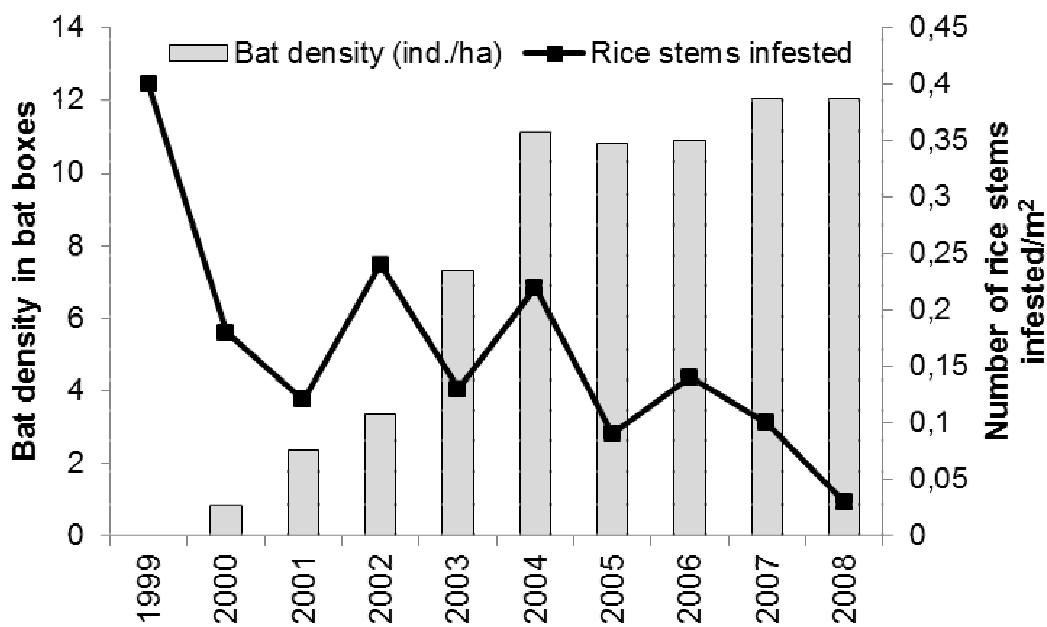


Figure 5.- Temporal patterns of striped rice borer damage (rice stems infested/ m^2 during the first peak of the borer: $r = -0.73$, $p < 0.01$, $n = 10$.) and bat densities (ind./ha: $r = 0.95$, $p < 0.001$, $n = 10$) on Buda Island since the bat boxes were installed.

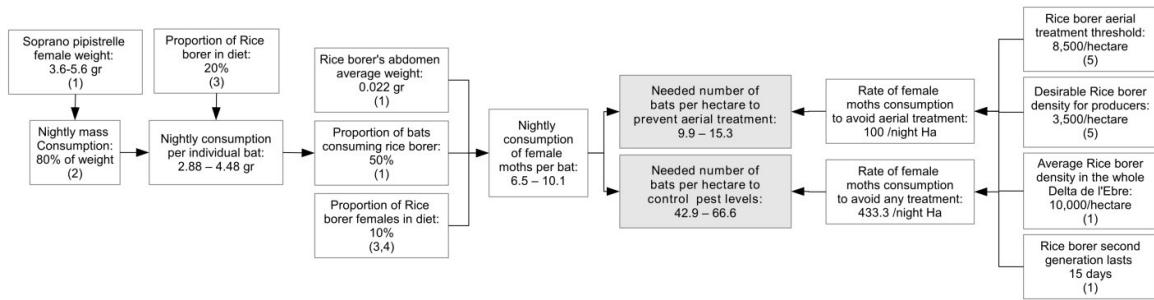


Figure 6.- Estimate of the soprano pipistrelle *Service Providing Unit* required to keep pest density below the target threshold during the second peak of rice borer activity. The Service Providing Unit is expressed as the number of adult individuals per hectare. Where (1) Source: data collected by the authors during the study; (2) Lowest estimation from Kurta et al. (1989); (3) Conservative untested assumption (4) Female borer population is proportional to number of stems damaged since they lay the eggs on the rice stems, the effect of reducing the male borer population on the next generation of larvae is uncertain and therefore is not considered in this model; (5) Source: thresholds used by the ADV Delta de l'Ebre (Ebre Delta Crop Defence Association of 2013).