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Habitat use by foraging bats (Mammalia: Chiroptera) in southern Italy determined by broad-band acoustic surveys and radiotracking

Danilo Russo

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with the requirements of the degree of Ph.D. in the
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Abstract

I developed acoustic identification methods and studied habitat use in a speciose bat community from southern Italy. I studied echolocation calls from 23 Italian bat species and devised multivariate discriminant functions used for habitat use studies. I described diagnostic *Pipistrellus kuhlii* social calls. I demonstrated sympatry of *Pipistrellus pipistrellus* and *P. pygmaeus* in Italy. I also examined resting frequencies from *Rhinolophus euryale* and *R. mehelyi*: juveniles called at lower frequencies than adults. The implications of these findings for acoustic investigations are discussed.

Habitat selection at the community level was determined by acoustic surveys. Activity was highest over rivers and lakes. *M. daubentonii* and *M. capaccinii* were affected by wind, and temperature influenced *Hypsugo savii* activity. *P. kuhlii*, *P. pipistrellus* and *Tadarida teniotis* showed no significant habitat preference. Rivers were selected particularly by *Myotis* bats and *Miniopterus schreibersii*.

Habitat selection by *R. euryale* was studied by radiotracking. This species selected broadleaved woodland. Urban, open areas and conifer plantations were avoided.

The results have clear implications for bat research and conservation in southern Italy and other Mediterranean areas. Acoustic identification by quantitative echolocation and social call analysis promises to be a valuable means to investigate habitat use by bats with high intensity echolocation calls in speciose Mediterranean bat communities. Rivers and riparian vegetation need to be adequately protected and managed. Habitats managed or created by humans were also important and should therefore feature in conservation plans. I recommend avoiding the clearance of continuous, large areas of woodland, and not planting conifers. Urbanisation is a threat to *R. euryale* and other sensitive species, and should be limited in areas of special value for the bat fauna. Linear landscape elements such as tree lines and hedgerows should also be preserved.

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Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree. Any views expressed in the dissertation are those of the author and in no way represent those of the University of Bristol.

The dissertation has not been presented to any other university for examination either in the United Kingdom or overseas.

Signed:

(Danilo Russo)

Date:

13 September 2001

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One. - Introduction

1.1. – Field methods to assess habitat use by foraging bats: a brief review

1.1.1. – Studying habitat use in bats

Much of this thesis is devoted to assessing habitat use by Italian bats in order to provide information for conservation plans. In this section I will provide a general introduction to the field techniques used to study habitat use by bats.

It is well known that bats show preferences in habitat exploitation (chapter six). Foraging habitat preferences by bats may be assessed in different ways. Measuring a specific descriptor of foraging activity – such as the capture success, when capture devices are employed (e.g. Lumsden and Bennett, 1995), or the number of bat passes in acoustic surveys (e.g. Vaughan *et al.*, 1997a) – in different habitats makes it possible to carry out a comparison between habitats: in this way, the relative use of habitats is evaluated and their importance for a species, or a community, may be highlighted. Radiotracking techniques appear especially suitable for making a comparison between used and available resources: if the use of a certain habitat is disproportional to availability, then selection occurs (e.g. Johnson, 1980; Aebischer *et al.*, 1993).

A variety of field techniques to investigate habitat use by bats is available today. None can be regarded as the best, since each may prove more or less effective in relation to the species studied.

Basically, methods may be classified into four main categories: a) ultrasound detection; b) visual observation; c) Use of mist-nets and harp-traps; and d) radiotracking

1.1.2. – Ultrasound detection

In the last decades, bat detectors (e.g. Ahlén,1980; 1991; Jones, 1993; Pettersson, 1999; Parsons *et al.*, 2000) have become increasingly popular instruments (Plate 1.1). Their basic function is to convert ultrasonic signals emitted by bats into audible sounds. Whenever a flying bat calls within the detector's range, its presence is revealed because its ultrasonic signals are detected and made audible. Effectiveness in revealing the presence of a bat depends upon the detector's sensitivity (Waters and Walsh, 1994; Parsons, 1996), call intensity (e.g. Jones and Waters, 1995), habitat structure in which monitoring is performed (Parsons, 1996), and distance between and relative positions of source and receiver. By directly listening to the sound output to the bat detector, or analysing it with appropriate sound analysis hardware and software (Plate 1.1), the researcher may also attempt species identification. I will not deal with the problem of identifying species by their ultrasound now, since this is covered in chapter five.

Three types of detectors have been widely used to study the European bat fauna (e.g. Ahlén, 1981; 1990; Zingg, 1990; Vaughan *et al.*, 1997 a; 1997b; Parsons and Jones, 2000): heterodyne detectors, frequency division detectors, and time expansion devices. Most recently, direct ultrasound sampling has also been successfully used (Pettersson, 1999; Jones *et al.*, 2000; Parsons and Jones, 2000). Each of these methods has pros and cons, which may be summarised as follows.

1.1.2.1. – Heterodyne detectors

Heterodyne detectors are the oldest devices applied to the study of bat echolocation. To study bat calls, Pierce and Griffin (1938) first employed a heterodyne device developed for hearing insect ultrasonic emissions. While their device only used one internal oscillator, modern heterodyne detectors employ two oscillators, and should therefore more precisely be termed 'super-heterodyne' detectors (Parsons *et al.*, 2000).

In heterodyne detectors, a first oscillator generates a frequency (whose value is set by the user) and such a signal is mixed with that from the bat, picked up by the detector microphone. Two resulting frequencies are thus obtained, one determined by the sum of the bat call and the oscillator frequencies, the other from their difference: a filter suppresses the latter, and the former is again mixed with the high-frequency signal generated by a second (constant frequency) oscillator. Again, two peak frequencies are produced, one well above the human hearing range, the other within it. In this way, the signal is made audible (Parsons *et al.*, 2000).

By modulating the first oscillator frequency, the user may identify the frequency value (± 5kHz) where the incoming signal is null: the corresponding value, read on a display, is often close to the call frequency of highest energy. Because heterodyne detects only calls falling into a narrow range set by the user (it is a narrow-band method), all bat passes pitched outside this range are missed. Moreover, heterodyne retains no information on duration, frequencies and on the frequency-time course of the call (Parsons *et al.*, 2000), so heterodyne-processed calls are not suitable for quantitative analysis.

1.1.2.2. – Frequency division

The incoming signal frequency is divided by a ratio set by the user, and this lowers the frequency to audible values (Parsons *et al.*, 2000). The system is broad-band, i.e. all incoming frequencies are processed, and therefore especially useful to detect all bat passes regardless of frequency (e.g. Vaughan *et al.*, 1997a). Although measurements may be taken from frequency-divided calls (e.g. Zingg, 1990), call structure is not completely preserved because only the harmonic with the highest energy is tracked (Parson *et al.*, 2000). Moreover, the signal-to-noise ratio is quite low, and clear recordings are difficult to obtain.

1.1.2.3. – Time-expansion

This is a very advantageous way of recording ultrasound, because the call structure is completely preserved and may be analysed in detail (Pettersson, 1999). Input calls are digitised at a high sampling rate, and then 'slowed down', i.e. converted into a waveform at a lower output rate (Pettersson, 1999; Jones *et al.* 2000; Parsons *et al.*, 2000). Apart from the high cost of time-expansion devices, a major problem lies in the fact that it is not possible to time-expand continuously: a sample of a certain duration (e.g. 2s, 3s, 12s) is time-expanded by a given factor (generally x10), and during the output phase (lasting e.g. 20s, or 30s if 2s, or 3s respectively are sampled with a x10 factor) no further signal may be expanded. This reduces the actual operating time of the detector (Jones *et al.*, 2000; Parsons *et al.*, 2000).

1.1.2.4. – Direct ultrasound sampling

Computer technology has recently made it possible to sample ultrasound signals without lowering their frequency. A laptop computer fitted with a data acquisition card sampling at frequencies > 330kHz is needed (Pettersson, 1999; Jones *et al.*, 2000). In this way, unlike time expansion, continuous sampling is possible; the system, however, is not as robust and portable as the others so far mentioned (Jones *et al.*, 2000).

1.1.2.5. – Conducting acoustic surveys

A basic aim of an acoustic survey is to assess use by bats of several sites or habitat types, performing limited (e.g. bats classified to family, or genera) or no identification. Bat passes may be counted to index bat activity, but it should be stressed that in no case may these express population densities, because several bat passes may originate from the same individual (Thomas and West, 1989). When identification is not a main objective in the study protocol, even large numbers of observers, not necessarily experienced in bat identification, may be employed to investigate bat activity on the large scale. Comparison of overall bat abundance between different habitats may be carried out, and predictions about suitability of further sites for foraging bats may be made (Walsh *et al.*, 1995; Walsh and Harris, 1996a; 1996 b). When feasible, species recognition makes it possible to collect detailed information on habitat use by one or more species (e.g. McAney and Fairley, 1988; Rachwald, 1992; Rydell *et al.*, 1994; Vaughan *et al.*, 1996; Shiel and Fairley, 1998; Waters *et al.*, 1999). Recordings may be carried out when stationary at point transects (e.g. McAney and Fairley, 1988; Rachwald, 1992) , or while moving along transects on foot, by bike or car (e.g. Ahlén, 1990; Walsh *et al.*, 1995, Walsh and Harris, 1996a; 1996b; Vaughan *et al.*, 1997a). Devices which automate the monitoring and recording process while unattended have also been used successfully (e.g. Downs and Racey, 1999; O'Donnell, 2000). The choice of a specific sampling protocol strictly depends on the study aim and situation, and special attention should be paid to it. Species emitting calls which are faint (e.g. *Plecotus*) or deeply affected by atmospheric attenuation (e. g. Griffin, 1971; Lawrence and Simmons, 1982) because of their high frequency (e.g. rhinolophids) may be underrepresented in acoustic surveying, and alternative methods should be adopted to study habitat use.

1.1.3. – Visual observation

A given bat species may be observed with a night-scope, filmed or photographed, and data on its foraging strategies acquired in this way, provided the species may be unambiguously recognised under such circumstances (e.g. Jones and Rayner, 1988; Kalko and Schnitzler, 1989; Arlettaz, 1996; Siemers and Schnitzler, 2000). However, this approach is of little use in habitat use studies. Otherwise, the bats may be captured and fitted with reflective bands and chemoluminescent tags (Racey and Swift, 1985; Barclay and Bell, 1988; Barataud, 1992; Pavey and Burwell, 2000). These tags have a short life-span and tend to be groomed off by bats, so data must be generally collected within a few hours from tagging; moreover, especially in cluttered habitats, the bats may easily move out of sight (Barataud, 1992). These techniques are more useful to document behavioural patterns than habitat use; in some cases, however, habitat use was successfully assessed with reflective bands (Racey and Swift, 1985) or light-tags (Pavey and Burwell, 2000), but large numbers of observers need to be involved in field data collection.

1.1.4. – Use of mist-nets and harp-traps

Mist-nets and harp-traps make it possible to capture bats. Mist-nets are less expensive and easy to transport, but they must be checked continuously and the bats quickly removed from them. If a net is left unattended, the bat gets very tangled, may be injured and will probably damage the net by biting it. A variety of techniques exists about where and how to erect nets in order to maximise capture success (e.g. Kunz and Kurta, 1988; Dilks *et al.*, 1995; Jones *et al.*, 1996). Nets erected at water sites, under bridges over water or partly hidden by the surrounding riparian vegetation, generally provide good capture rates. In the field work carried out for the present thesis, I captured many bats by setting the nets beside cattle troughs in woodland sites far from other potential drinking sites (Plate 1.2). Capture rates in woodlands and other non-aquatic habitats are generally lower. In a study on a forest bat species from New Zealand, *Chalinolobus tuberculatus* (Sedgeley and O'Donnel,1999), the bats were caught in canopy-height mist-nets (Dilks *et al.*, 1995) in order to be fitted with radio-tags: the capture success in this habitat was < 0.01 bats per net hour. The results provided by mist-netting may therefore be influenced by the habitat features. Susceptibility to capture varies among species, and this may bias habitat use analysis. Finally, when mist-nets are set at the same site and in the same position bats learn how to avoid them (Kunz and Brock, 1975). Harp-traps are more expensive and less portable than mist-nets. Harp-traps have a reduced intercept surface, and are more effective when set by the roost exit or along fly pathways than when placed in foraging sites. The traps are far less stressful for bats than mist-nets, and removal is easy (Plate 1.2). Sedgeley and O'Donnell (1996) harp-trapped *Chalinolobus tuberculatus* at tree-roosts, and concluded that the method did not adversely affect bats. Harp-traps are more efficient than mist nets for the capture of many rhinolophid and hipposiderid species (see Kingston *et al.*, 2000).

In spite of the above described limitations, when a strict, well-controlled sampling protocol is adopted, captures may still provide valuable information on habitat use by bats (e.g. Lumsden and Bennett, 1995).

1.1.5. - Radiotracking

Bats may be fitted with miniaturised radio-transmitters and tracked by means of a receiver (Plate 1.3). This technique has been widely applied to bats to determine their home-range, activity patterns, habitat use and roost selection (e.g. Fenton, 1983; Fenton and Rautenbach, 1986; Jacobsen *et al.*, 1986; Krull *et al.*, 1991; Jones and Morton, 1992; Duvergé, 1996; Bontadina *et al.*, 1999a; 1999b, Entwistle *et al.*, 1996; Sedgeley and O'Donnell, 1999; Smith 2000).

Although radiotracking may seem a rather invasive method, the impact on bats is actually not significant when the tag weight is low enough. Aldridge and Brigham (1988) estimated that *Myotis yumanensis* lost 5% of manoeuvrability when a tag 5% of their body mass was applied. In order to minimise the influence of the tag on the bat's normal behaviour, a general rule is therefore that the tag should not exceed 5% of the bat body mass (Aldridge and Brigham, 1988). Hickey (1992) found no significant difference between the foraging success of tagged and non-tagged *Lasiurus cinereus* (the tags weighed ca. 3% of the bat body mass).

Details on tagging and tracking are given in the Material and Methods section of chapter seven.

Radiotracking makes it possible to collect data from individuals; the researcher's final aim, however, is to extrapolate features of a certain population, or species from individual data. Information from very small samples may be strongly affected by aberrant, or sex and age related behaviour (Rabinowitz, 1997). In order to avoid such possible bias, a sample of at least 10 bats should be considered (Bontadina *et al.*, 1999a).

1.2. – The Italian bat fauna

1.2.1. - Overview

All 31 European bat species occur in Italy (Tab. 1.1), on the basis of both historical and recent records (Lanza, 1959; Lanza and Finotello, 1985; Lanza and Agnelli, 1999; Russo and Jones, 2000). The latest species documented for the Italian territory, *Pipistrellus pygmaeus* (Leach, 1825), was discovered in 1998 during field work for this thesis (Russo and Jones, 2000; see also chapter three). Bats are the most speciose mammalian order in Italy, accounting for about 30% of the total number of native mammal species and outnumbering even rodent species (Fig. 1.1). Tab. 1.1 shows the status of Italian bat species for Italy (Bulgarini *et al.*, 1998) and in a global context

(IUCN, 2000). It is worth stressing that in some cases conservation status for Italy was defined on the basis of very little information, because no systematic survey of bat populations has ever been carried out in the country; only very recently has bat research started to be taken seriously. At a global scale, Italy has 8 species considered Vulnerable, and 11 Low risk – nearly threatened species (Tab. 1.1).

I will now review the main facts concerning occurrence, range and conservation status of Italian bat species.

1.2.2. – Rhinolophidae

Four out of the five European rhinolophids currently occur in Italy (Lanza, 1959; Lanza and Agnelli, 1999). *Rhinolophus blasii* may be extinct (Bulgarini *et al.*, 1998). The only confirmed observations of this species date back to the eighteenth century and refer to the Trieste province (north-east Italy; Ninni, 1878; De Beaux and Dal Piaz, 1925). All other records of *R. blasii* for Italy are considered uncertain (Lanza, 1959).

Rhinolophus ferrumequinum and *Rhinolophus hipposideros* (Plate 1.4) are present in all Italian regions (Lanza, 1959) and are quite widespread. A number of colonies of the former are anecdotally said to have undergone a numerical decline (Vernier, 1997). *R. hipposideros* is defined as 'widespread but uncommon' in the Red List of Italian Fauna (Bulgarini, 1998), and classified as endangered. *Rhinolophus euryale* (Plate 1.5) is defined as 'Vulnerable' by Bulgarini *et al.* (1998), but information about its status is very poor, and according to my personal observations, in southern Italy it is less frequent than *R. ferrumequinum* and *R. hipposideros*. The level of threat for this species is likely to have been underestimated. *Rhinolophus mehelyi* (Plate 1.5) shows a very unusual distribution: apart from a few, relatively old records for the Puglia region (south-west Italy), and some others, unconfirmed (Amori *et al.*, 1993), for Sicily (Lanza, 1959), the only known colonies occur in Sardinia (Mucedda *et al.*, 1994-95). Sardinian colonies are very large, up to several thousand bats (Mucedda *et al.*, 1994-95). Some Sardinian individuals show a peculiar orange-coloured fur (Mucedda, 1994).

1.2.3. – Vespertilionidae

The majority of bat species occurring in Italy are vespertilionids. Among them is the commonest Italian bat (Lanza, 1959), the Kuhl's pipistrelle *Pipistrellus kuhlii* (Plate 1.6). Especially abundant at lower elevations (Vernier and Bogdanowicz, 1999), it shows a high degree of synanthropy and may be commonly seen roosting and foraging even in large cities.

Three other species from the genus *Pipistrellus* occur in Italy: the cryptic common (*P. pipistrellus*) and soprano (*P. pygmaeus*) pipistrelles (Russo and Jones, 2000), and *P. nathusii*. Uncommon in the country – especially in the south (Lanza, 1959), *P. nathusii* was recently observed breeding in the north (Martinoli *et al.*, 2000). Also common in the country is Savi's pipistrelle (*Hypsugo savii*; Plate 1.6), a species formerly known as *Pipistrellus savii* (Bonaparte, 1837), then ascribed to a genus on its own – *Hypsugo* – on the basis of morphological characters intermediate between *Pipistrellus* and *Eptesicus* (Horáček and Hának, 1985-86).

Among the *Myotis* bats (Tab. 1.1, Plate 1.7), *M. dasycneme* should only be cited as accidental for Italy. Indeed, only one specimen was found in the country – a female from Trento captured in May 1881 (Lanza, 1959), now held at the Museo Zoologico de La Specola (Florence). *Myotis bechsteinii* is one of the rarest bat species in Italy, and few recent observations exist (Vergari *et al.*, 1998). The case of *Myotis brandtii* is controversial. Its Italian range is practically unknown, and the close resemblance to its sibling species *M. mystacinus* may have hindered researchers in shedding light on the species' actual occurrence and range. *M. brandtii* certainly occurs in Tuscany (one specimen from the Monte Amiata; Lanza, 1959), and according to Vernier (1997) also in north-east Italy (but these records are considered uncertain by Lanza and Agnelli (1999)). The bats reported for the Abruzzo National Park by Zava and Violani (1995) as *M. brandtii* were in fact *Myotis daubentonii* (this erroneous record was also included for the European distribution in Mitchell-Jones *et al.* (1999)). More recently, however, Issartel (1998) captured *M. brandtii* at the Abruzzo National Park: three females, which were identified on the basis of dental features (Baagøe, 1973), and a male whose penis morphology made possible a confident identification (Issartel, *in litteris*).

A final point on Italian *Myotis* bats concerns mouse-eared bats from Sardinia. Strelkov (1972) described them as belonging to the subspecies *M. blythii omari* (the peninsular subspecies is *M. b. oxygnathus*), and Felten *et al.* (1977) attributed them to the north-African subspecies *M. b. punicus*. Ruedi *et al.* (1990) and Arlettaz (1995) showed that Sardinian mouse-eared bats from the island all belong to a single species – *M. myotis* – and that the sibling *M. blythii* does not occur on the island. New molecular evidence (Castella *et al.*, 2000), however, suggests that Sardinian mouse-eared bats belong to a third new cryptic taxon separated from *M. myotis* and *M. blythii*, also occurring in Corsica and north-Africa and provisionally named *Myotis* cf *punicus* Felten, 1977.

Plecotus austriacus is found in most Italian regions (Swift, 1998). Altough *P. auritus* (Plate 1.8) is often reported to occur only in the north of the country (Crucitti, 1990; see also distribution in Swift, 1998), in fact some records from areas further south also exist (Abruzzo: Zava and Violani, 1995; Campania: Sbordoni, 1963; Puglia: Marsico, 1998-99). A recent analysis of roost distribution from 1990 (GIRC, *in press*) showed that *Plecotus auritus* roosts are confined prevalently to north-western Italy, whereas those of *Plecotus austriacus* are found from the northern Apennine ridge southwards; roosts are occasionally shared by both species. The three *Nyctalus* species from continental Europe (*N. noctula, N. leisleri, N. lasiopterus*) all occur in Italy. Little information is available on their distribution, because their tree-dwelling habits make them difficult to observe. Italy is home to one of the most southerly European breeding populations of *Nyctalus noctula*, which during summer roosts in various *Platanus europaeus* tree cavities bordering avenues in Cervia, Emilia-Romagna (D. Scaravelli, *pers. comm.*). Records of *Nyctalus leisleri* (Plate 1.8) are scattered throughout the country (Lanza, 1959; Dondini and Vergari, 1995; Mucedda, 1997). *Nyctalus lasiopterus* is very rare in Italy (Vergari *et al.*, 1997), and the latest record concerns specimens found in bat-boxes in Tuscany (Dondini and Vergari, 2000). The analysis of droppings from these bats showed that *Nyctalus lasiopterus* sometimes preys on birds as well as insects, a unique case among European bats (Dondini and Vergari, 2000).

The barbastelle (*Barbastella barbastellus*) is regarded as very rare in Italy (Bulgarini *et al.*, 1998). Until 2000, only six roosts (none of which was a nursery) were known from north-east Italy (GIRC, *in press*). In 1998, Issartel (1998) captured a few lactating females of barbastelle in the Abruzzo National Park territory. Russo (2000) confirmed the occurrence of breeding barbastelles (lactating females were captured at the end of July 2000) in the Park and in August 2000 Russo and Jones identified a tree roost by radiotracking a post-lactating female (Plate 1.9). The site from the Abruzzo Park is the only barbastelle breeding site known for Italy. The maternity roosts are probably located within the same capture area, and if this is confirmed they are located at the highest elevation so far recorded in Europe (over 1200 m a. s. l.; previous record from the Czech Republic was 1100 m a. s. l., Schober and Grimmberger, 1997).

The Schreiber's bat (*Miniopterus schreibersii*; Plate 1.10) is, morphologically, a peculiar vespertilionid, and some authors classify it in a separate family, Miniopteridae (e.g. Lanza and Agnelli, 1999). Highly gregarious, in Italy colonies may consist of several thousand bats (Lanza, 1959). A rather strictly cave-dwelling bat, one of the main threats to its status comes from caving, and protection of caves is therefore fundamental for preservation of colonies (Bulgarini *et al.*, 1998).

1.2.4. – Molossidae

The only molossid bat occurring in Europe (Schober and Grimmberger, 1997), *Tadarida teniotis* (Plate 1.10) has long been regarded as a rare species in Italy. Lanza (1959) defined it as 'infrequent in the country, although not as rare as commonly believed'. In fact, the species seems rather common in Italy, and may be encountered even in large cities (e.g. Russo and Mastrobuoni, 1998). No information on population size, however, is available to date, and roosts are practically unknown. The tendency to fly high up and its roosting habits (it often roosts in crevices in inaccessible cliffs; Arlettaz, 1990) may have contributed to the underestimation of its occurrence. For example, in the Campania region (south-west Italy), the main study area for my thesis, the species was recorded (Russo and Forgione, 2000) over a century after the previous observation (Costa, 1843; Monticelli, 1886). Indeed, the characteristic, audible echolocation calls from *T. teniotis* may commonly be heard over much of the Italian territory.

1.2.5. – Why so many species?

The occurrence of such a high bat biodiversity may be explained by ecological and biogeographical considerations. The Italian territory covers a wide latitudinal range and shows a heterogeneous mosaic of habitats, offering a vast range of ecological conditions (from the arid, sun-burnt plains of Puglia to rocky environments at high altitudes in the steep, snowy Alps; from the sclerophyllous *Quercus ilex* woodlands to beech (*Fagus sylvatica*) woodlands. As is typical of the Mediterranean region, in Italy many habitats have been determined by the profound, centuries-old influence of humans on the territory (Blondel and Aronson, 1999): habitats such as terraced olive (*Olea europaea*) groves, chestnut (*Castanea sativa*), woodlands, and Apennine secondary grasslands, just to mention some, were all created by humans but nowadays they are an important part of the semi-natural landscape of Italy. The variety of habitats offers conditions for species with very different ecological demands. Italy's central position in the Mediterranean has made colonisation possible by many vertebrate species migrating from the surrounding regions. Such simple considerations help explain the high species richness of the Italian fauna, and in our case, of bats.

1.3. – Bat research in Italy

The most famous Italian bat scientist of all time was certainly Lazzaro Spallanzani (1729-1799), one of the founders of experimental biology. His famous experiments on bats first suggested a relationship between the way bats orientate during flight and their audition. His observations led to the monograph (Spallanzani, 1794) '*Lettere sopra il sospetto di un nuovo senso nei pipistrelli*' (i.e. 'Letters about the possibility of an unknown sensorial capacity in bats'). Following this, many Italian scientists took an interest in the natural history of bats (see Crucitti, 1990 for a review). In 1837, Prince Carlo Luciano Bonaparte described a new European bat species, the now endangered long-fingered bat *Myotis capaccinii.*

Among the classical studies on Italian bats from the nineteenth century, it is worth citing the fundamental review of bat distribution by Gulino and Dal Piaz (1939) and the thorough description of bat morphology, biology and distribution by Lanza (1959). The latter first showed that *Plecotus auritus* and *Plecotus wardii* (= *austriacus*) occurred sympatrically in Italy, and were separate species (Swift, 1998).

After Lanza, and until a few years ago, few bat researchers were active in the country. In the sixties, one of them, G. Dinale conducted the only long-term ringing project so far carried out in the country (e.g. Dinale, 1960; Dinale and Ghidini, 1966). Indeed, most academic researchers neglected bat ecology, conservation biology and distribution, regarded as pursuits for amateurs. Professional research interests mainly focused on bat anatomy, cytology and histology (Crucitti, 1989), fields traditionally considered more rewarding in the interests of a career in Italian universities. As a result, by the eighties, in spite of the ever growing general concern about bat conservation in Europe, practically no information on bat ecology relevant to the protection and management of these mammals in Italy was available (Stebbings, 1988). In the nineties, a new interest in bat studies developed in Italy, especially concerning distributional studies (e.g. Zava and Violani, 1992; Vernier, 1997). The EC 'Habitats Directive' further encouraged bat research in the country. A considerable problem, however, was still the almost complete lack of communication among researchers. In March 1998, the first Italian Bat Conference was held in Castell'Azzara, Tuscany (Dondini *et al.*, 1999).

On the occasion of the second Italian Congress of Theriology, held in 1998, bat researchers met to found the Italian Chiroptera Research Group (*Gruppo Italiano Ricerca Chirotteri, GIRC*). The main aims of GIRC are to promote research and conservation of bats in Italy and to improve cooperation among zoologists involved in bat studies. I was among the GIRC founders, and currently am the national vice-coordinator of this association.

In order to get a preliminary picture of bat distribution and conservation status in Italy, one of the projects launched by GIRC members was to set up a database of all information collected on bat roosts from 1990 to 1999. The first results of this work were presented at the VIII European Bat Research Symposium (Russo and Scaravelli, 1999), and led to a paper in the Symposium Proceedings (GIRC, *in press*). The roost database will be constantly updated in order to extend the knowledge available, particularly for the areas of the country so far insufficiently covered. The GIRC also runs a web site where group news and activities are publicised.

1.4. – Legal protection and conservation needs of Italian bats

1.4.1. – Bats and the Italian law

The first Italian law to declare bats as protected mammals dates back to 1939 (T.U. 1939, art. 38). At that time, bats were already regarded as predators of pest insects and therefore considered important for agriculture. The law, still in force, protects the animals but not their roosts or foraging sites. All too few people know that bats are protected (even police and Forestry Corps officers are often unaware of the fact), and it is far from rare for bats roosting in houses to be exterminated. Given to a generally excessive tolerance for crimes towards wildlife, and negligence in the application of laws, offenders are seldom prosecuted. The recent European 'Habitats Directive' and the subsequent Italian law that has made the EC directive legally applicable to the Italian territory (D.P.R. 357, 8th Sept., 1997) has slightly improved the legal backgroumd to conservation. First, the fact that the occurrence of just one bat species of particular conservation value (as stated in the EC directive) allows a site to be legally protected has increased the conservation significance of bats in the country and attracted funds for bat conservation projects.

Second, this law forbids disturbance to wintering bats and alteration or destruction of breeding sites. This seems a considerable step forward, and in theory should help conservationists to carry out effective protection actions. In practice, there is much confusion about what should be considered a breeding 'site'. Local government institutions (the so-called 'Regioni' and 'Province') have in some cases claimed that a 'site' is an area rather than, for example, a building where bats roost: as a result, the interpretation of the law does not ensure legal protections of roosts (especially in the case of buildings). Italian bureaucrats and true wildlife conservationists are seldom good friends.

Finally, and surprisingly, no penalty for offenders of the above cited 1997 law in fact exists. As a result, when a bat hibernaculum is disturbed or a breeding site destroyed, the law is violated, but there may be no penal consequences! To date, Italian laws do not mention protection of bat foraging habitats. In 2001, the administrative procedure through which Italy will become part of the European Bat Agreement is in progress. The improvement of the framework for bat conservation is urgently needed.

1.4.2. – People's attitude towards bats

Bats are not popular animals in Italy, and this hostility goes back a long way. The Romans regarded bats as evil creatures, and nailed them to their house doors to keep demons away. In the Middle Ages and later, the Christian culture and art did not help much: angels were always portrayed with bird wings, demons with bat wings.

Many popular beliefs feature bats as evil and disgusting creatures. In southern Italy, for example, country folk still believe that bats are reincarnations of witches, ready to eat people's eyes, get tangled in their hair, and even believe that bat urine causes your hair to fall out. I shall not go into people's reactions on meeting someone who studies bats! The legend that bats fasten onto people's hair is surprisingly common even in cities and among well-educated people. Although one might be tempted to dismiss these stories as folklore, they still do serious harm to the cause of bats, and there is an urgent need for education of the public.

1.4.3. – Conservation needs of Italian bats

The public attitude is not the most important threat to Italian bats. Bats are put at risk by several factors (e.g. Bulgarini *et al.*, 1998), all common to other areas of Europe (e.g. Stebbings, 1988; Mitchell-Jones *et al.*, 1999; Hutson *et al.*, 2001). These factors may be summarised as follows:

1) disturbance, destruction or alteration of roosts

2) Incorrect forest management practices, often involving the logging of large, continuous woodland parcels and the removal of old or dead trees with subsequent loss of tree-roosts for tree-dwelling species;
3) Alteration and destruction of foraging biotopes;
4) Widespread use of pesticides in agriculture;
5) Treatment of wooden components of buildings used as roosts with toxic substances;
6) Urbanisation;
7) Pollution

The Italian territory is karstic, characterised by thousands of natural caves, several of which are large and spectacular. There are also large artificial underground sites, frequently of archaeological interest. Caving is becoming increasingly popular in Italy. While some cavers are interested in the study and protection of cave organisms, many are not, and explorations of caves where large hibernating or breeding bat colonies occur are now common. In some cases, explosives are used (illegally) to remove obstructions and open new underground routes. People are often unaware that bat colonies need to be left undisturbed. The *R. euryale* roost where the radiotracking work described in this thesis was conducted was advertised in the most popular Italian wildlife magazine, *Airone*, in an article (Barnabei, 1998) entitled 'Caves to explore. Walking in the heart of the ancient aqueduct'. In some cases even researchers may constitute a source of disturbance. Bats are sometimes ringed without a well-defined scientific aim. Some old-fashioned researchers still think that the only bat suitable for study is a dead one. Soon after discovering *P. pygmaeus* in Italy (chapter three), I was approached by a famous, elder researcher who asked me whether I had 'kept, *legally or not*, any *pygmaeus* specimen for *my personal collection*'. As a matter of fact, I have no personal collection. It must be said, however, that bat protection is gaining ground in the academic world, and most bat projects currently in progress in Italy are in the interests of conservation.

Information on the status and ecology of bats from Italy is scarce (Bulgarini *et al.*, 1998), and studies as the basis for designing chiropteran conservation plans for Italy (Stebbings, 1988), and more generally the Mediterranean region (Hutson *et al.*, 2001) are urgently needed.

Conservation plans should include education programmes as well as scientific research. Moreover, politicians must be more active in transforming the potential guidelines for bat protection identified by conservationists into concrete actions.

1.5. – Thesis overview and conventions used

My main aim in this thesis was to study habitat requirements of Italian bats, an aspect of bat ecology about which little or nothing was known when my investigation started. I based my work in southern Italy, an area in which bat fauna was poorly studied. Specifically, I aimed to obtain information which could be applied to bat conservation in the country. In chapters two, three, four, five, six and seven I present and discuss the original research data obtained in this study. Each of these chapters has been organised as a separate paper and may be read independently from the others.

When I began my work, echolocation calls by Italian bats had never been studied systematically and most acoustic identification studies were attempted using heterodyne detectors. No description of social calls from Italian bats and their potential application to bat identification was available. I began practising with sound analysis and its applications to identification by studying social calls in the commonest Italian bat, the Kuhl's pipistrelle (*Pipistrellus kuhlii*). I explored the possibility of using such signals to tell *P. kuhlii* apart from other sympatric pipistrelle species. This appeared especially useful when identification based on echolocation calls was uncertain. This study is dealt with in chapter two.

My PhD project started soon after DNA analysis had unequivocally demonstrated that the bat formerly known as *Pipistrellus pipistrellus* corresponded to two cryptic species (Barratt *et al.*, 1997), difficult to identify according to morphology but relatively easy to tell apart from their echolocation calls (Jones and Parijs, 1993). Hence, I explored the hypothesis that echolocation call frequencies by Italian 'common pipistrelles' followed a bimodal distribution: if they did, then the two species were sympatric in Italy too. Because pipistrelle social calls are diagnostic (Barlow and Jones, 1997a; 1997 b) I also examined recordings of social calls from Italian pipistrelles to further support evidence from echolocation call analysis. The results are described in chapter three.

In the course of the project, my interest in echolocation call design and adaptive meaning grew further, and led me to start a side-project on the influence of sex, age and body size on echolocation calls of two poorly studied rhinolophids, the Mediterranean and the Mehely's horseshoe bats (*Rhinolophus euryale* and *R. mehelyi*). To record *R.*

mehelyi, I worked in Sardinia where the only large population of this species occurs. The results are described in chapter four.

Acoustic surveys represent a very effective approach to identifying key foraging habitats. Acoustic identification in a speciose community such as that occurring in my study area is particularly challenging because many bat species emit similar echolocation calls and species recognition should therefore be conducted with special care and effective methods. To conduct my habitat use investigation, I needed to devise a method for acoustic identification. Hence, I applied discriminant function analysis (Vaughan *et al.*, 1997b) to large samples of echolocation calls from individuals of known identity of most Italian bat species to obtain an objective, quantitative identification. This method also offers the advantage of measuring how good the identification of each species is (this is expressed as % identification rate; Vaughan *et al.*, 1997b). Chapter five deals with this aspect of my study.

The acoustic identification methods devised allowed me to investigate the importance of foraging habitats for bats of southern Italy. Ten habitat types, among the most representative of southern Italy, were surveyed. Results are dealt with in chapter six.

Because rhinolophids call at high frequencies, their calls are largely influenced by atmospheric attenuation (e. g. Griffin, 1971; Lawrence and Simmons, 1982); therefore, as anticipated in section 1.1.2.5., acoustic surveys are unlikely to reveal their presence. Radiotracking appeared to be a more promising method for a habitat use study.

Among the three rhinolophids occurring in my study area, I decided to concentrate my attention on *R. euryale*, which appears the least common. My choice was also stimulated by the absence of previous studies on habitat selection by this bat in other areas. The best alternative to acoustic survey in studying this species habitat preferences was radiotracking, which I conducted on a *R. euryale* colony located in northern Campania. The results obtained are discussed in chapter seven.

In chapter eight, the results obtained are discussed further in order to stress several key points which should be considered in conservation plans for Italian bats.

In this thesis, the nomenclature of bats previously known as *Pipistrellus pipistrellus* follows Jones and Barratt (1999). Hereafter, pipistrelles of the 45 kHz phonic type (Jones and Parijs, 1993) are referred to as *P. pipistrellus*, and those of the 55 kHz phonic type are termed *P. pygmaeus*. The nomenclature adopted for all other bat species follows Schober and Grimmberger (1997).

All measurements are given following the metric system. In all statistical tests, significance was set at $p < 0.05$.

To allow the non-Italian reader to locate the Italian places mentioned in this thesis, a political map of Italy is shown in Plate1.11.

One final point should be dealt with. Because my research was conducted on the Italian territory, hereafter I will refer to 'Italian bats', 'Italian bat fauna', etc. Political and ecological geography, however, often diverge. Indeed, the latitudinal range covered by the Italian peninsula determines marked differences between the south and most northern regions. Undoubtedly, the southern area where habitat use was investigated (mainly Campania and a few sites in southern Lazio) is, in terms of ecology and landscape structure, 'Mediterranean' before being 'Italian', closer to the Peloponnesian, in Greece, than to Italian regions such as Lombardy and Piedmont.

Tab. 1.1. Bat species occurring in Italy, their status in the country (after Bulgarini *et al.*, 1998) and in a global context (after the 2000 IUCN Red List of Threatened Species). Taxonomy and vernacular nomenclature (except *Pipistrellus pygmaeus*) after Mitchell-Jones *et al.* (1999). Categories: CR = Critically endangered, EN = Endangered, VU = Vulnerable, LR = Low risk, DD = Data deficient. Subcategories: A2c (under VU) = identifies threshold levels of population reduction (at least 20%) predicted in the future, based on decline in area of occupancy, or extent of occurrence and/or quality of habitat; nt (under LR) = Near Threatened; i.e. taxa which do not qualify for Conservation Dependent, but which are close to qualifying for Vulnerable; lc (under LR) = least concern, i.e. taxa which do not qualify for Conservation Dependent or Near Threatened. Status of '*Pipistrellus pipistrellus*' was defined before presence of *Pipistrellus pygmaeus* in Italy was recorded.

Species	Italian vernacular name	Status in Italy	Global status
Rhinolophus blasii Peters, 1866	Ferro di cavallo di Blasius	CR	LR: nt
Rhinolophus euryale Blasius, 1853	Ferro di cavallo euriale	VU	VU:A2c
Rhinolophus ferrumequinum (Schreber, 1774)	Ferro di cavallo maggiore	VU	LR:nt
Rhinolophus hipposideros (Bechstein, 1800)	Ferro di cavallo minore	EN	VU:A2c
Rhinolophus mehelyi Matschie, 1901	Ferro di cavallo di Mehely	VU	VU:A2c
Barbastella barbastellus (Schreber, 1774)	Barbastello	EN	VU:A2c
Eptesicus nilssonii (Keyserlin and Blasius, 1839)	Serotino di Nilsson	DD	LR:nt
Eptesicus serotinus (Schreber, 1774)	Serotino comune	LR	LR:lc
Hypsugo savii (Bonaparte, 1837)	Pipistrello di Savi	LR	LR:nt
Myotis bechsteinii (Kuhl, 1817)	Vespertilio di Bechstein	DD	VU:A2c
Myotis blythii (Tomes, 1857)	Vespertilio minore	VU	LR:lc
Myotis brandtii (Eversmann, 1845)	Vespertilio di Brandt	DD	LR:lc
Myotis capaccinii (Bonaparte, 1837)	Vespertilio di Capaccini	EN	VU:A2c
Myotis dasycneme (Boie, 1825)	Vespertilio dasicneme	DD	VU:A2c
Myotis daubentonii (Kuhl, 1817)	Vespertilio di Daubenton	VU	LR:lc
Myotis emarginatus (E. Geoffroy, 1806)	Vespertilio smarginato	VU	VU:A2c
Myotis myotis (Borkhausen, 1797)	Vespertilio maggiore	VU	LR:nt
Myotis mystacinus (Kuhl, 1817)	Vespertilio mustacchino	VU	LR:nt
Myotis nattereri (Kuhl, 1817)	Vespertilio di Natterer	EN	LR:nt
Nyctalus lasiopterus (Schreber, 1780)	Nottola gigante	EN	LR:nt
Nyctalus leisleri (Kuhl, 1817)	Nottola di Leisler	VU	LR:nt
Nyctalus noctula (Schreber, 1774)	Nottola comune	VU	LR:nt
Pipistrellus kuhlii (Kuhl, 1817)	Pipistrello albolimbato	LR	LR:lc
Pipistrellus nathusii (Keyserling and Blasius, 1839)	Pipistrello di Nathusius	VU	LR:lc
Pipistrellus pipistrellus (Schreber, 1774)	Pipistrello nano	LR	LR:nt
Pipistrellus pygmaeus (Leach, 1825)	Pipistrello pigmeo	-	-
Plecotus auritus (Linnaeus, 1758)	Orecchione comune	LR	LR:lc
Plecotus austriacus (J. B. Fischer, 1829)	Orecchione meridionale	LR	LR:lc
Vespertilio murinus Linnaeus, 1758	Serotino bicolore	DD	LR:lc
Miniopterus schreibersii (Kuhl, 1817)	Miniottero	LR	LR:nt
Tadarida teniotis (Rafinesque, 1814)	Molosso del Cestoni	LR	LR:lc

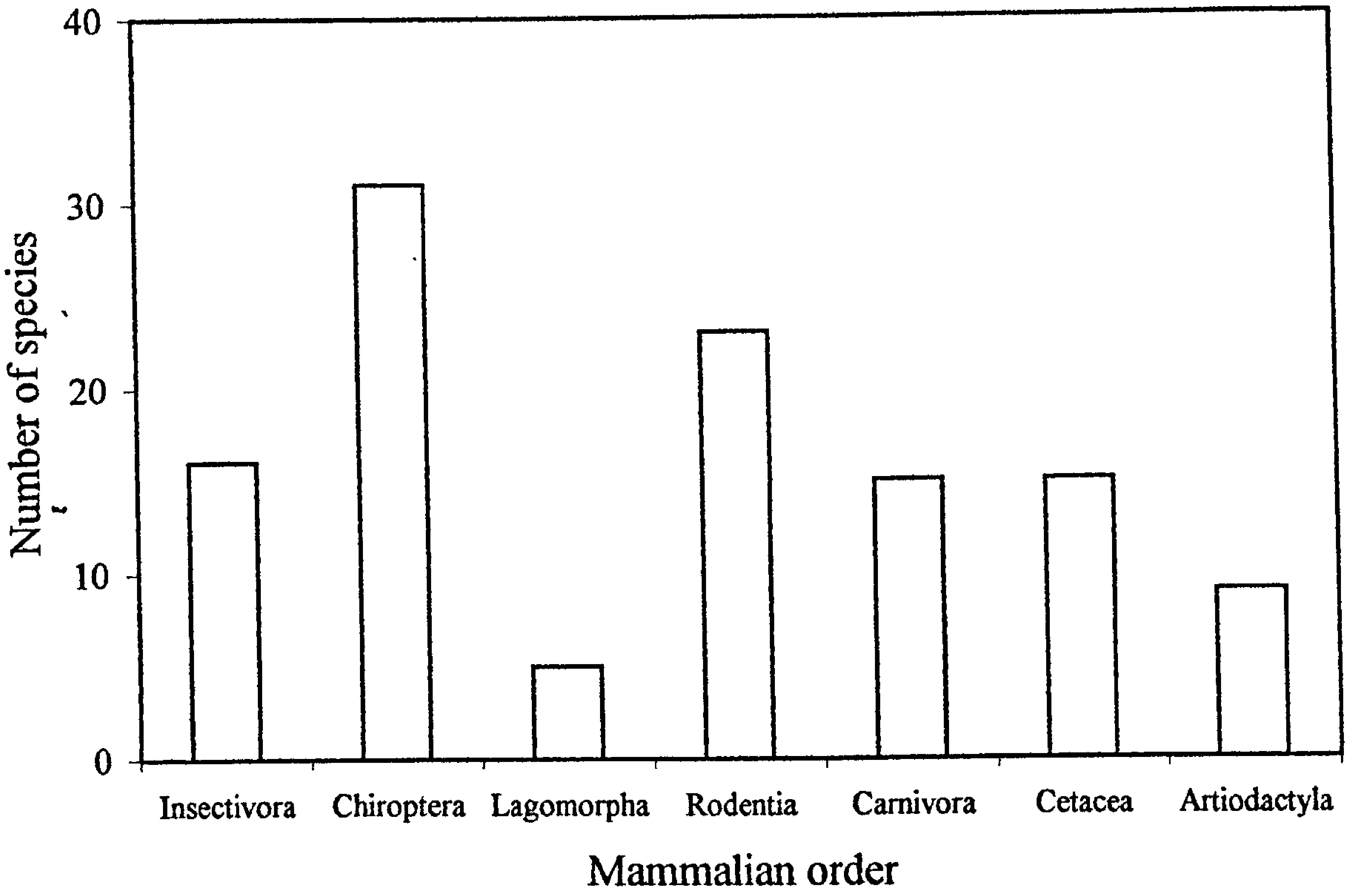

Fig. 1.1. Number of mammalian species occurring in Italy (after Amori *et al.*, 1999). Species introduced after 1900 are not included.

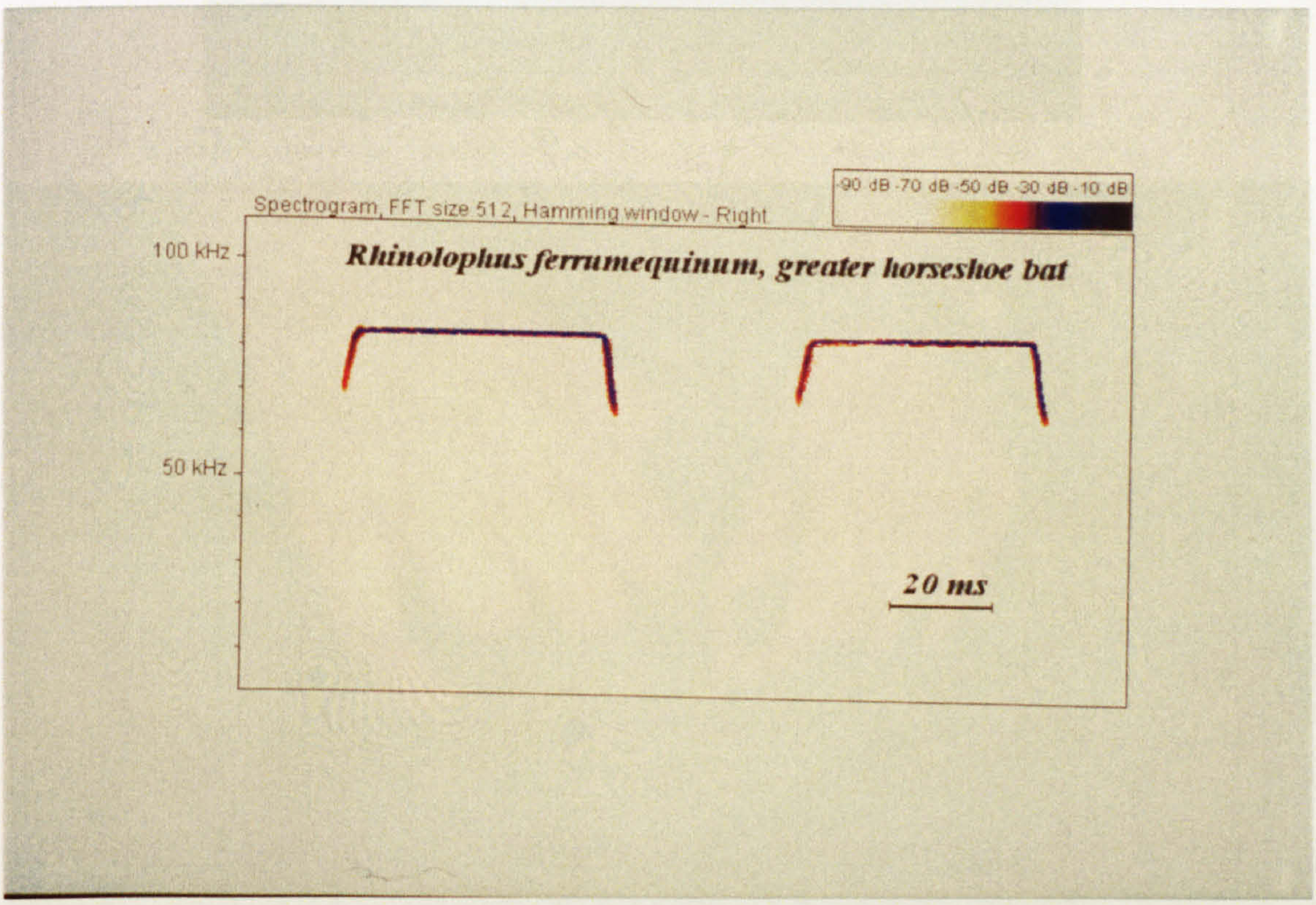

Plate 1.1. Above: the equipment used in this study to record bat calls: S25 bat detector, PUSP and Sony Walkman WM D6C *(photograph by S. Viglietti)*. Below: typical output (spectrogram) from a sound analysis software (BatSound).

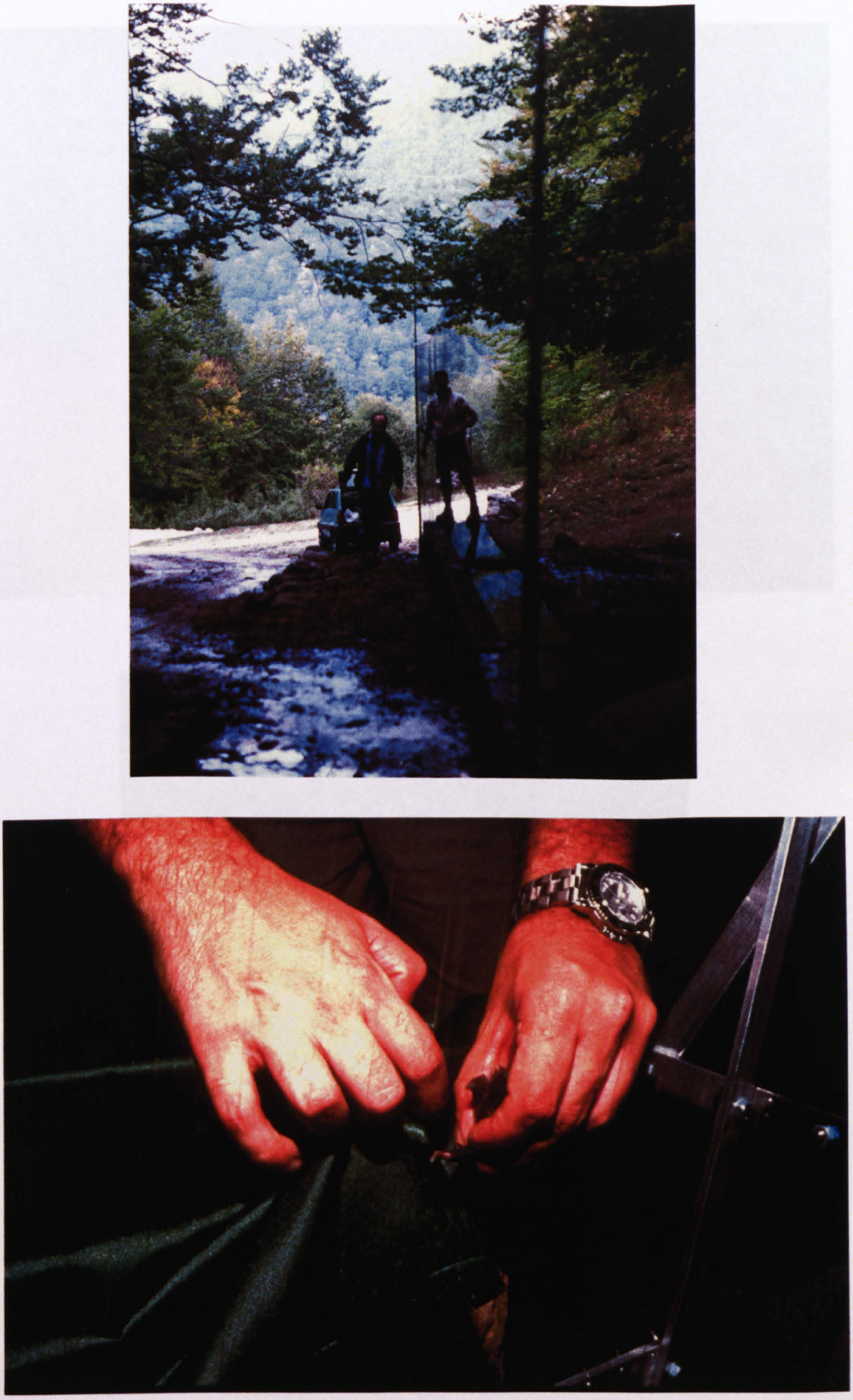

Plate 1.2. Above: A mist-net placed by a cattle trough. Abruzzo National Park, September 2000 *(photograph by L. Vacca)*. Below: removing a bat from a harp-trap. Benevento province, July 2000 (*photograph by S. Viglietti)*.

Plate 1.3. Above: a Holohil LB-2 tag. Below: radiotracking *Rhinolophus euryale*. Benevento province, July 2000 (*photographs by G. Mastrobuoni*).

Plate 1.4. Above: *Rhinolophus ferrumequinum (photograph by S. Viglietti)*. Below: *Rhinolophus hipposideros (photograph by G. Bulfoni).*

Plate 1.5. Above: *Rhinolophus euryale (photograph by S. Viglietti)*. Below: *Rhinolophus mehelyi* from a Sardinian cave *(photograph by M. Mucedda)*.

Plate 1.6. Above: a juvenile *Pipistrellus kuhlii (photograph by D. Scaravelli)*. Below: *Hypsugo savii (photograph by G. Jones)*.

Plate 1.7. Two of the 10 *Myotis* species occurring in Italy: *Myotis myotis* (above) and (below) *Myotis daubentonii (photographs by G. Jones).*

Plate 1.8. Above: *Plecotus auritus*. Below: *Nyctalus leisleri (photographs by G. Jones).*

Plate 1.9. Above: a female *Barbastella barbastellus* caught at the Abruzzo National Park (August 2000). Below: the same bat after tagging *(photographs by G. Jones).*

Plate 1.10. Above: *Miniopterus schreibersii (photograph by S. Viglietti)*. Below: *Tadarida teniotis (photograph by M. Mucedda)*.

Plate 1.11. A physical and political map of Italy.

Two. – The social calls of Kuhl's pipistrelles *Pipistrellus kuhlii* (Kuhl, 1817): structure and variation

2.1. – Summary

The aim of this study was to describe the structure of social calls produced by Kuhl's pipistrelles (*Pipistrellus kuhlii*). Bats foraging around street lamps in the Campania region (southern Italy) were recorded. Calls were produced by bats during chases and, similarly to those of *Pipistrellus pipistrellus* and *P. pygmaeus*, were probably used to repel conspecifics from hunting sites. Calls were often composed of three components, lasted on average 34 ms and contained most energy at about 17 kHz. A positive correlation was found between the frequencies of maximum amplitude of echolocation and social calls. Social calls from two adjacent populations differed in their peak frequencies, and possible hypotheses for this variation are given. The structure of *P. kuhlii* social calls was compared with those of *P. pipistrellus* and *P. pygmaeus*. Kuhl's pipistrelle calls lasted longer and showed lower values of minimum and peak frequencies. These differences can help to discriminate between field recordings of *P. kuhlii* and *P. pipistrellus/pygmaeus* where these species occur sympatrically.

Russo, D. and Jones, G. (1999). The social calls of Kuhl's pipistrelles *Pipistrellus kuhlii* (Kuhl, 1819): structure and variation (Chiroptera: Vespertilionidae). *J. Zool., Lond.* 249 : 476-481, is based on this chapter.

2.2. – Introduction

The Kuhl's pipistrelle *Pipistrellus kuhlii* (Kuhl, 1819) is a small vespertilionid with a body mass of 5-10g and a forearm of 31-37 mm (Schober and Grimmberger, 1997). It is distributed through south and south-west Europe, including the Balkans and the Mediterranean islands, extending northwards to Switzerland, France and Austria, eastwards to the Caucasus (Schober and Grimmberger, 1997) and southwards to Africa (Corbet, 1978).

P. kuhlii echolocation search phase calls are 8-12 ms in duration (Schnitzler *et al.*, 1987) and consist of a short frequency-modulated (FM) component followed by a terminal part whose frequency is almost constant (CF). The latter corresponds to the frequency of highest energy, generally 35-40 kHz (Schnitzler *et al.*, 1987; Ahlén, 1990; Barataud, 1996). Call structure varies depending on whether the bat flies in cluttered or uncluttered habitats (Kalko and Schnitzler, 1993).

Like many other bat species, bats from the genus *Pipistrellus* also emit vocal signals for communication (Ahlén, 1981; 1990; Miller and Degn, 1981; Fenton, 1985). These calls may serve different functions: *Pipistrellus pipistrellus*, for example, produces social calls in an aggressive context (Barlow and Jones, 1997a), songflight calls by males to attract females to the roost in the mating season (Lundberg and Gerell, 1986; Gerell-Lundberg and Gerell, 1994; Barlow and Jones, 1997b), and distress calls to attract conspecifics and probably to incite other bats to mob predators (Russ *et al.*, 1998).

Social calls can be used to discriminate between different pipistrelle species: significant differences exist between the structures of advertisement calls of *P. pipistrellus* and *Pipistrellus nathusii* (Barlow and Jones, 1996), and even the two cryptic species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Jones and Parijs, 1993; Park *et al.*, 1996; Barlow *et al.* 1997; Barratt *et al.*, 1997; Jones and Barratt, 1999) can be separated by examining the structure of their songflight and social calls (Barlow and Jones, 1997b).

Although *P. kuhlii* is known to emit social calls (Ahlén, 1990; Barataud, 1996), no detailed description of these signals has been reported so far.

In the present study I 1) provide a detailed analysis of social calls emitted by foraging Kuhl's pipistrelles, 2) test the hypothesis that bats emitting echolocation calls with higher peak frequencies also produce social calls with higher frequencies of maximum energy, and 3) investigate whether there are geographical differences in social call structure between two populations less than 100 km apart.

I also discuss the possibility of discriminating *P. kuhlii* in flight from other pipistrelle species producing similar echolocation calls (*P. pipistrellus*, *P. nathusii*) by examining social calls.

2.3. – Methods

2.3.1. – Field recordings

From August to October 1998, I recorded social calls emitted by foraging bats in the Campania region, S. Italy (Long. 14°15'E, Lat. 40°50'N). *P. kuhlii* is the commonest bat species in this area. Almost all calls analysed were recorded in the Benevento and Naples provinces, about 70 km apart. Only one call was recorded in the Salerno Province.

I made recordings near street lamps, where *P. kuhlii* commonly forages (Haffner and Stutz, 1985/6) and seems to take advantage of group hunting (Barak and Yom-Tov,1989).

The habitats occurring at the recording sites in the Benevento province were arable land, olive groves and vineyards, while in the Naples province I carried out recordings in urban areas and suburbs with a mosaic of buildings, small cultivations and gardens. In order to avoid pseudo-replication (Hurlbert, 1984), I made recordings at lamps located at least 2 km apart, and considered for analysis only one call sampled at each site. In this way only one signal for each bat was represented in the sample.

Recordings were made via the high-frequency output of an S25 bat detector (Ultra Sound Advice, London) connected to a Portable Ultrasound Processor (Ultra Sound Advice, London) which sampled at a rate of 448 kHz and time-expanded (10x) a 2 ms sequence of calls. The resulting sequence, lasting 20 s, was then replayed and recorded on Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C. The S25 microphone has a sensitivity of –57dB±3dB (ref. 1V/µbar) from 20-120 kHz.

I could compare *P. kuhlii* social calls with those by British *P. pipistrellus* described by Barlow and Jones (1997b) as an identical equipment was used in both studies.

2.3.2. – Sound analysis

The recordings were analysed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). I adopted a sampling frequency of 44100 samples/s, with 16 bits/sample; a 512 pt. FFT with a Hamming window was used for analysis.

For each social call, I measured the following parameters (Plate 2.1): the number of components to the call (*nocomp*), the total duration of the call (*totdur*), the minimum (*fmin*) and maximum (*fmax*) frequencies of the call, and the mean frequency of highest energy (*freq*) by measuring the peak frequency of each component and calculating the average. Duration was measured from oscillograms, and all other parameters were taken from spectrograms.

2.3.3. – Identification of Pipistrellus kuhlii

P. kuhlii is the most abundant bat species in all localities where I carried out recordings. Its presence in ten of them was also confirmed by hand-netting the bats soon after ultrasound recordings had been taken.

In all cases, I attributed the social calls recorded to *P. kuhlii* only when they were produced by bats emitting FM-CF echolocation calls with a frequency of maximum amplitude of 36-41.5 kHz (Plate 2.2). *P. kuhlii* echolocation calls show a wider range of peak frequencies (Zingg, 1990); however, the criterion I adopted eliminated any possible risk of confusion with *P. pipistrellus,* as the lowest values of peak frequencies reported for this species from continental Europe (Zingg, 1990) and Great Britain (Vaughan *et al.*, 1997) is 41.6 kHz, and Zingg (1990) indicates values of peak frequency for *P. kuhlii* and *P. pipistrellus* which overlap within the range 41.6-44.8 kHz .

In most cases, the bats I considered for the present study emitted echolocation calls peaking below 40 kHz. No individuals of *P. pipistrellus* were captured in the study areas.

P. nathusii also emits echolocation calls very similar to those produced by *P. kuhlii* (Zingg, 1990); however, this species is uncommon in southern Italy (Lanza, 1959) and is not known to occur in Campania.

2.3.4. – Statistical analysis

An Anderson-Darling test applied to the parameters measured on social calls and on peak frequencies of echolocation calls revealed that they did not conform to normal distribution, and normality was not obtained through data transformation. Therefore, I used non-parametric tests for univariate analyses: a two-tailed Mann-Whitney test was applied to test for differences between medians of each parameter, and a Spearman's rank coefficient was used to explore correlation between the peak frequencies of echolocation and social calls. As multivariate techniques are robust to departures from normality (Dillon and Goldstein, 1984), I also employed a quadratic discriminant analysis with cross validation to try to separate call samples from the two study areas and a MANOVA to obtain values for Wilk's λ. Analyses were performed with Minitab release 11.0.

2.4. – Results

2.4.1. – Pipistrellus kuhlii social calls

P. kuhlii foraged at all recording sites, as confirmed by the numerous feeding buzzes (Griffin *et al.*, 1960) they produced. Two or more bats flew by the street lamps, and in most cases I could observe them chasing while emitting social calls. Due to their low frequencies, these signals were distinctly audible to the unaided ear. A total sample of 50 social calls, each from a different bat, was analysed.

Fig. 2.1 shows a typical social call of *P. kuhlii*. Thirty out of 50 Kuhl's pipistrelles produced three-component social calls, although calls with 2, 4 and – in one case – 5 components were recorded (Fig. 2.2). Calls lasted approximately 34 ms, peaked at 16.6 kHz and showed a mean frequency bandwidth of 26.7 kHz (Tab. 2.1).

I found a significant positive correlation between *freq* and the peak frequency of the echolocation call preceding the social call ($n = 40$, $r_s = 0.342$, $p < 0.05$). The median values of peak frequency measured on the echolocation calls preceding the social calls did not differ significantly between Naples and Benevento (nNaples = 27, nBenevento = 13, $T = 126.0$, $p = 0.156$).

Of all variables measured on calls from the two study areas, only *freq* showed a significant difference, i.e bats from Benevento emitted social calls peaking at frequencies about 2 kHz higher than those from Naples (Tab. 2.2). A quadratic discriminant analysis with cross validation carried out on samples from the Benevento ($n = 22$) and Naples ($n = 27$) provinces could correctly classify 61.2 % of calls, but the model was not significant (Wilk's $\lambda = 0.79$, $F_{5,43}=2.2$, $p = 0.07$).

2.4.2. – Comparison between Pipistrellus kuhlii and P. pipistrellus/pygmaeus

Spectrograms of social calls of *P. kuhlii*, and of *P. pipistrellus* and *P. pygmaeus* all show more than one component (Fig. 2.1). Like *P. kuhlii*, *P. pygmaeus* also produces more frequently calls of 3 components, while *P. pipistrellus* emits 4-component calls (Barlow and Jones, 1997b). Social calls of *P. kuhlii* are longer than those of *P. pipistrellus* and *P. pygmaeus*, and show lower values of *fmin* and *freq* (Tab. 2.1). The difference in *fmax* appears considerable (about 4 kHz) only between *P. kuhlii* and *P. pipistrellus.*

2.5. – Discussion

2.5.1. – Pipistrellus kuhlii social calls

The social calls of *P. kuhlii* I describe were all emitted during chases in foraging grounds, and are therefore probably equivalent in function to those observed in *P. pipistrellus*, i.e. they are used for food patch defence (Racey and Swift, 1985, Barlow and Jones, 1997a). I also noticed that social calls were more frequent on cold nights, probably because insect density is reduced by low air temperatures (Williams, 1961). At low insect densities, *P. pipistrellus* performs most chases (Racey and Swift, 1985) and produces higher social call rates (Barlow and Jones, 1997a).

My study shows that bats emitting echolocation calls at higher peak frequencies also produce social calls with a higher frequency of maximum amplitude. This relationship does not occur in *P. pipistrellus/pygmaeus* (Barlow and Jones, 1997b).

As my data show no difference between echolocation call frequencies from the Naples and Benevento areas, the microgeographical variation observed in *freq* cannot be explained by the above discussed relationship between peak frequencies of echolocation and social calls. This difference could be due to mere random effects of natural variation. A further hypothesis, however, is that the Kuhl's pipistrelles from Naples emit social calls at lower frequencies as a result of an acoustic adaptation process aimed to reduce attenuation and degradation of propagating calls in a complex habitat such as the urban area, while bats from Benevento produce social calls with higher frequency as they forage in simpler, uncluttered habitats. The acoustic adaptation to habitat structure (Morton, 1975; Wiley and Richards, 1978) is known to occur at a microgeographical scale in several bird and mammal species (e.g. Galeotti *et al.*, 1996; Slobodchikoff *et al.*, 1998).

2.5.2. – Comparison between Pipistrellus kuhlii and P. pipistrellus/pygmaeus

Social calls of *P. kuhlii* show lower values of *freq* and *fmin* than those by British *P. pipistrellus* and *P. pygmaeus* (Barlow and Jones, 1997b). These differences are also likely to exist where these species occur sympatrically, as social calls of *P. pipistrellus* and *P. pygmaeus* from France, Portugal and Sweden, are very similar to those described for Britain (G. Jones, *unpublished data*). Values of *freq* are inversely related to body size between species, as *P. kuhlii*, which calls at the lowest frequencies, is larger than *P. pipistrellus/pygmaeus* and *P. pygmaeus*, which calls at the highest frequencies, is smaller than *P. pipistrellus* (Jones and Parjis, 1993; Barlow and Jones, 1997b). Larger bat species bear larger vocal tracts, and tend to produce echolocation calls at lower frequencies than smaller species (Barclay and Brigham, 1991; Jones, 1995; Vaughan *et al.*, 1997b). The negative relation to body size observed in pipistrelle social calls might be interpreted accordingly.

P. kuhlii calls also last longer than those by *P. pipistrellus* and *P. pygmaeus*, and an experienced listener might in many cases recognise this further difference by hearing the different "rhythm" of the consecutive call components in time-expanded calls, which appears slower in *P. kuhlii* (Barataud, 1996). Duration, however, is one of the most variable parameters in Kuhl's pipistrelle social calls because it is related to the number of components, and may overlap between social calls of *P. kuhlii* and *P. pipistrellus*.

The parameter *fmax* differs clearly only between the Kuhl's pipistrelle and *P. pipistrellus*. However, its measurement may not be reliable, as higher frequencies are more deeply affected by atmospheric attenuation (Griffin, 1971; Pye, 1980; Lawrence and Simmons, 1982).

A combined use of *fmin*, *freq*, and *totdur* measured on time-expanded social calls should help to discriminate between field recordings of unknown *Pipistrellus* whenever the identification based on echolocation calls is uncertain. The differences in frequency values observed are not sufficiently large to permit the usage of heterodyne detectors for a social call-based species identification.

In areas where *P. nathusii* and *P. kuhlii* occur sympatrically, a discrimination based on social calls should be easy to carry out as the former species emits typical advertisement and social calls constituted by a main part with a larger (5-7) number of components, and a final, higher-pitched trill (Barlow and Jones, 1996).

Tab. 2.1. Social call parameters from the 50 Kuhl's pipistrelles recorded in the present study, and from 24 *P. pygmaeus* and 22 *P. pipistrellus* after Barlow and Jones (1997b). Q1 and Q3 are the lower and upper quartiles.

	Pipistrellus kuhlii					*P. pygmaeus*	*P. pipistrellus*
Parameter	Mean ± S.D.	Range	Median	Q1	Q3	(mean ± S.D.)	(mean ± S.D.)
totdur (ms)	34.4 ± 8.31	21.2 – 56.3	33.5	28.0	39.0	24.9±3.48	30.5±3.46
fmin (kHz)	11.5±0.81	9.8-13.1	11.4	10.9	12.0	16.6±1.80	15.2±1.93
fmax(kHz)	38.2±4.50	29.5-49.2	38.5	35.7	40.8	39.5±5.69	34.4±4.91
freq (kHz)	16.6±2.70	13.5-24.6	16.2	14.2	18.3	20.8±1.56	19.8±2.53
nocomp	2.9±0.68	2-5	3	2.8	3.0	3.0±0.42	4.0±0.49

Tab. 2.2. Social call parameters of Kuhl's pipistrelles from the provinces of Naples (n = 27) and Benevento (n = 22), values of Mann-Whitney T statistic and corresponding levels of significance *p*. For each parameter the value from the Benevento sample is given below that from the Naples sample.

Parameter	Mean ± S.D.	Range	Median	Q1	Q3	T	*p*
totdur (ms)	33.5±7.04	22.6-52.0	33.8	26.6	38.8	293.5	0.952
	34.4±8.72	21.2-53.0	32.8	27.8	39.5		
fmin (kHz)	11.4±0.79	10.3-13.1	11.4	10.9	12.0	267.0	0.543
	11.5±0.86	9.8-13.1	11.4	11.1	12.0		
fmax(kHz)	37.4±4.91	29.5-48.6	37.3	33.4	40.7	229.5	0.177
	39.2±3.93	31.7-49.2	39.0	35.7	41.8		
freq (kHz)	15.7±2.20	13.6-22.9	15.4	14.0	17.3	161.5	0.003
	17.8±2.84	13.9-24.6	18.1	15.6	18.9		
nocomp	2.8±0.56	2-4	3.0	2.0	3.0	253.5	0.387
	3.0±0.79	2-5	3.0	2.7	3.2		

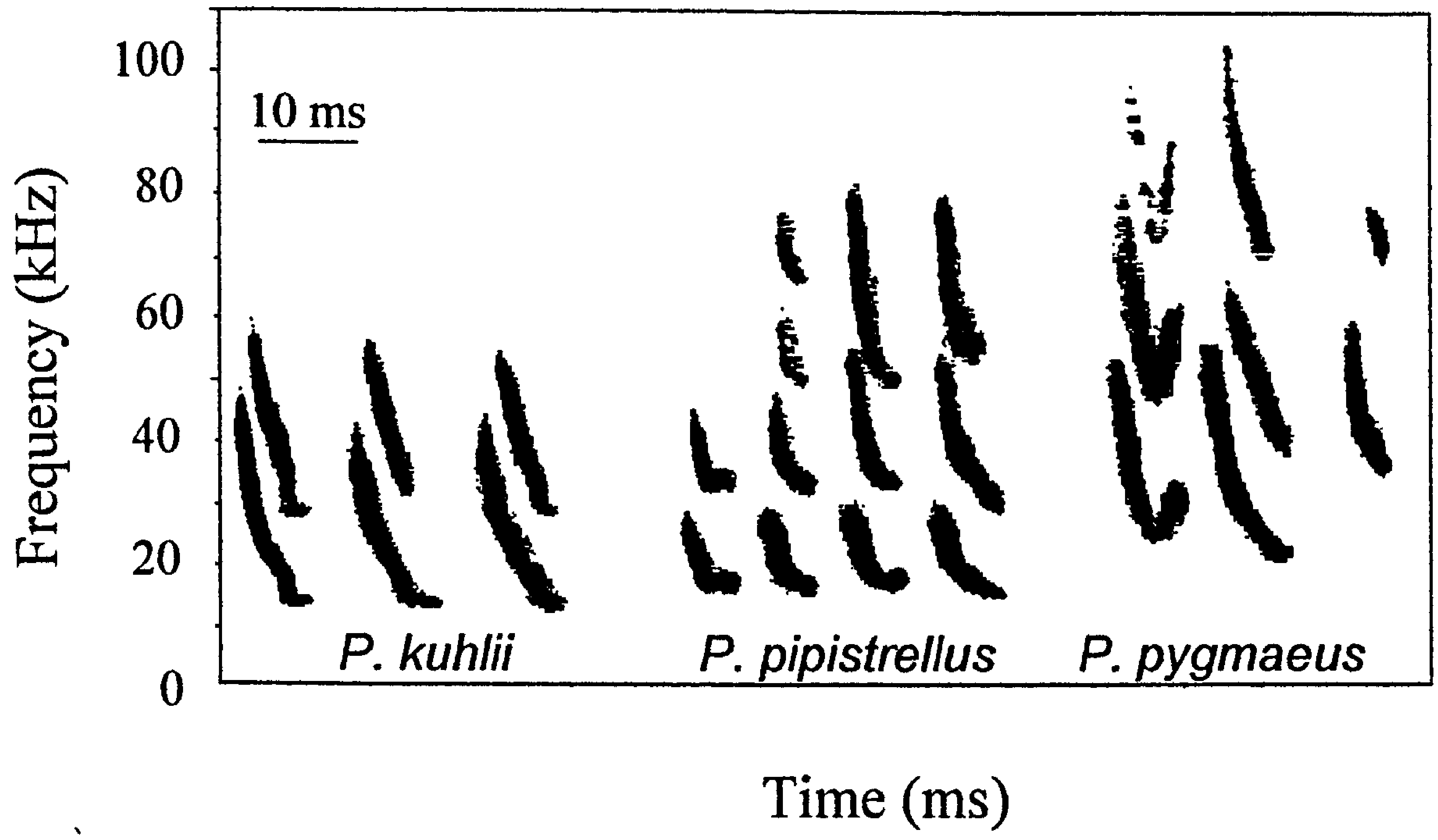

Fig. 2.1. Spectrograms of a social call of *Pipistrellus kuhlii*, *P. pipistrellus*, and *Pipistrellus pygmaeus*.

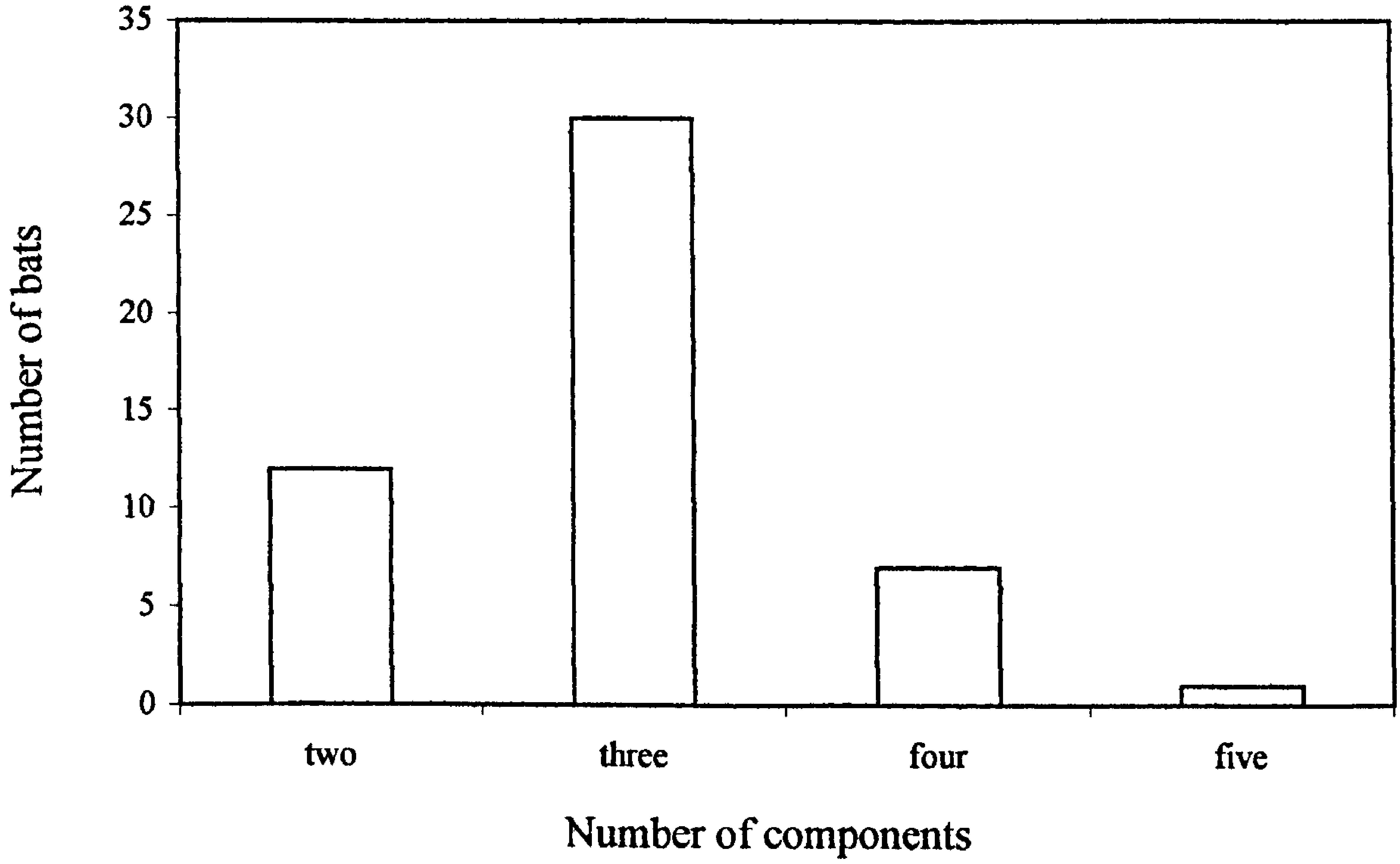

Fig. 2.2. Number of components of social calls emitted by 50 *Pipistrellus kuhlii*. Only one call from each bat was examined.

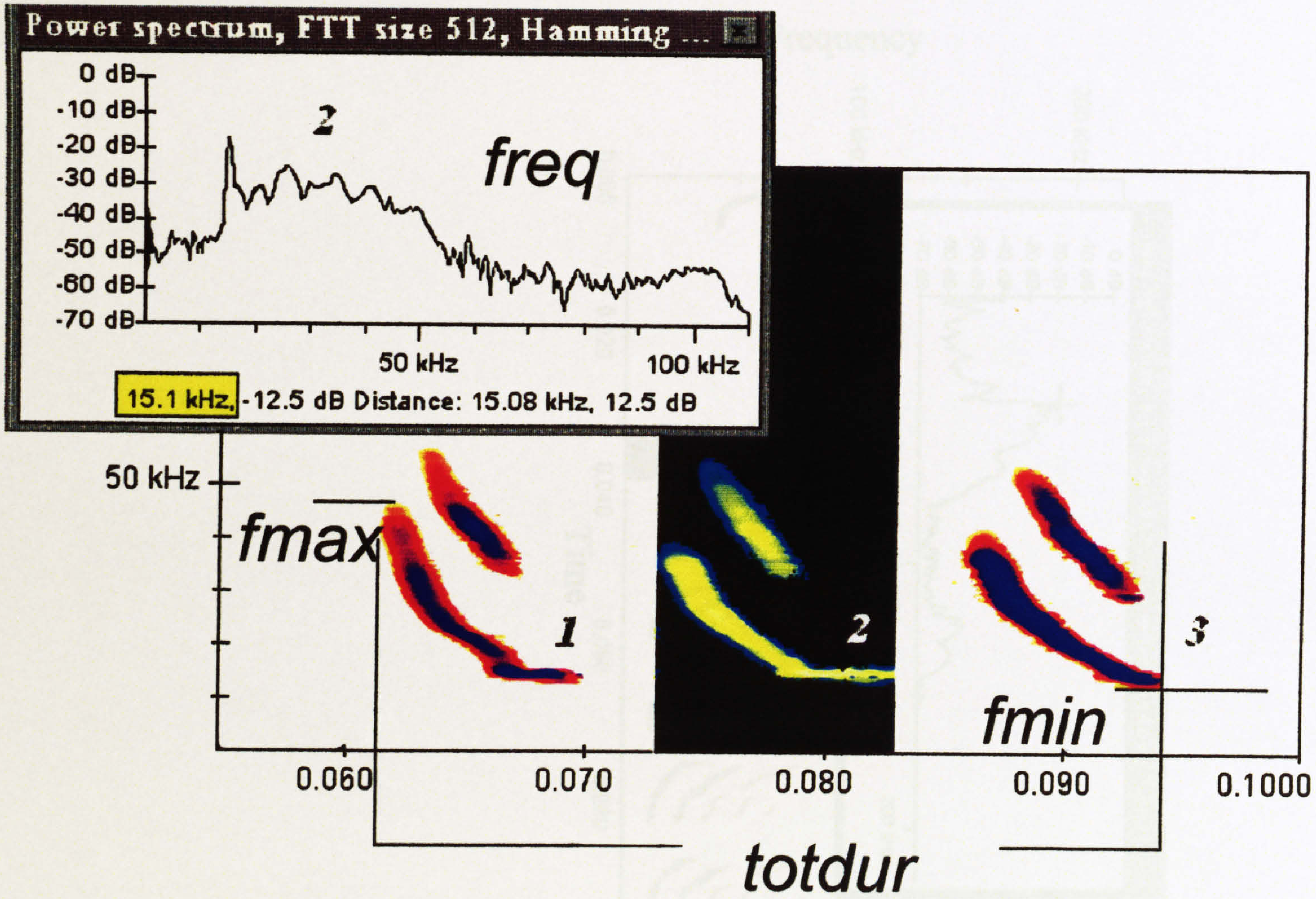

Plate 2.1. A BatSound output (spectrogram and power spectrum) showing variables measured from a Kuhl's pipistrelle social call. *totdur*: total duration; *fmax*, *fmin*: maximum and minimum frequencies; *freq*: frequency of maximum energy of each call component.

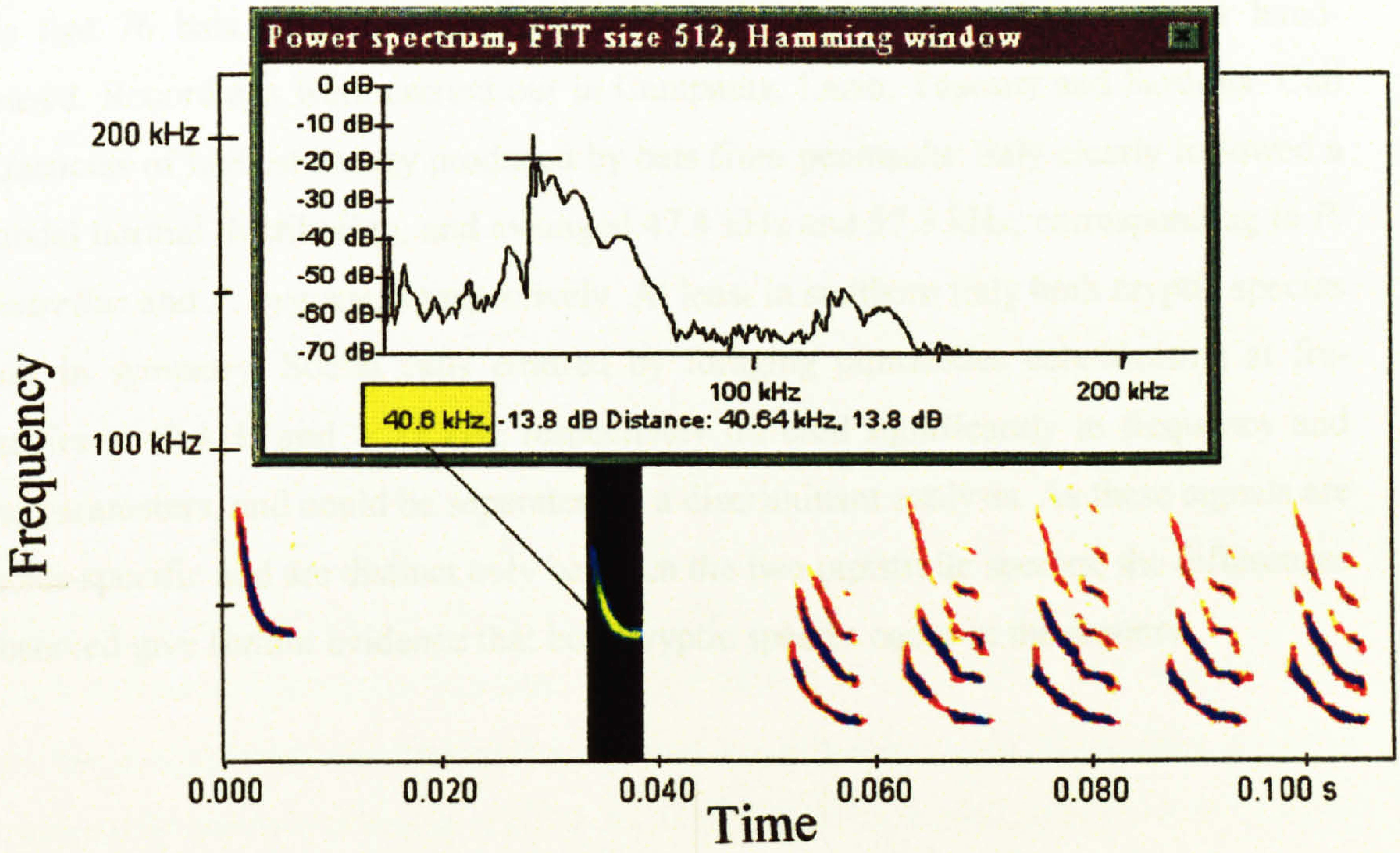

Plate 2.2. A BatSound output (spectrogram and power spectrum) showing a sequence of echolocation and social calls from *Pipistrellus kuhlii*. The social calls recorded were attributed to *P. kuhlii* only when they were produced by bats emitting echolocation calls with a frequency of 36-41.5 kHz (40.6 in this example).

Three. – Sympatry of *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* in Italy: evidence from echolocation and social calls

3.1. – Summary

I provide evidence based on the analysis of echolocation pulses and social calls that *Pipistrellus pipistrellus* and *P. pygmaeus* occur in Italy. I analysed the echolocation calls that 76 bats emitted when they were foraging, emerging from roost or hand-released. Recordings were carried out in Campania, Lazio, Tuscany and Sardinia. Call frequencies of highest energy produced by bats from peninsular Italy clearly followed a bimodal normal distribution, and averaged 47.4 kHz and 57.3 kHz, corresponding to *P. pipistrellus* and *P. pygmaeus* respectively. At least in southern Italy both cryptic species occur in sympatry. Social calls emitted by foraging pipistrelles echolocating at frequencies < 49 kHz and > 52 kHz respectively differed significantly in frequency and time parameters, and could be separated by a discriminant analysis. As these signals are species-specific and are distinct only between the two pipistrelle species, the differences I observed give further evidence that both cryptic species occur in the country.

Russo, D. and Jones, G. (2000). The two cryptic species of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) occur in Italy: evidence from echolocation and social calls. *Mammalia* 64 : 187-197, is based on this chapter.

3.2. Introduction

Until very recently, the common pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774) was regarded as one species. This bat emits FM-CF echolocation calls, i.e. calls consisting of a brief frequency-modulated (FM) part followed by a terminal component whose frequency is almost constant (CF) and contains the highest energy.

Zingg (1990) found that Swiss pipistrelles produced calls with a terminal frequency of either 44-46 kHz or higher than 50 kHz respectively. He hypothesised that this difference could be due to the existence of two types of hunting behaviour. Other authors had observed pipistrelle bats calling either below 49 kHz or above 50 kHz in several European regions (Ahlén, 1981; Miller and Degn, 1981, Weid and Helversen, 1987).

Jones and Parijs (1993) found that the frequency distribution for call frequencies of British pipistrelles follow a bimodal distribution, and that two phonic types, calling at about 46 kHz and 55 kHz, occur. The two pipistrelles use separate roosts (Jones and Parijs, 1993), do not form mixed mating groups (Park *et al.* 1996), show slight morphological differences (Jones and Parijs,1993; Barlow *et al.*, 1997; Plate 3.1), differ in their diets and habitat use (Barlow, 1997; Vaughan *et al.*, 1997a), and produce well-separable songflights - emitted by males for mate attraction - and social calls involved in food patch defence (Barlow and Jones, 1997a; 1997b). Social calls serve a species-specific communication function, and elicit no reaction from the other phonic type (Barlow and Jones, 1997a). Furthermore, the two phonic types are well separated on a genetical basis - their cythocrome *b* sequences of mtDNA diverge by over 11% (Barratt *et al.*, 1997).

The common pipistrelle therefore comprises two species, named *Pipistrellus pipistrellus* (the 45 kHz phonic type) and *Pipistrellus pygmaeus* (the 55 kHz one) according to the nomenclature proposal by Jones and Barratt (1999).

Little or nothing is known about the occurrence and relative abundance of the two cryptic species in many European countries. In this chapter I provide bioacoustical evidence that both cryptic species of pipistrelle occur in Italy. I test the hypotheses that echolocation calls in this region follow a bimodal distribution. Furthermore, as pipistrelle social calls are species-specific for each cryptic species (Barlow and Jones, 1997a; 1997b), I also test the hypothesis that social calls by Italian pipistrelles echolocating at frequencies of highest energy < 49 kHz and > 52 kHz respectively can be separated.

3.3. – Methods

3.3.1. – Field recordings

I recorded echolocation search calls under three conditions:

a) during emergence from roosts where the presence of pipistrelle bats had been established previously;

b) when bats were released from the hand in open habitats after being captured. The bats were mist-netted at foraging sites or while emerging from the roost or, on a few occasions, captured inside the roost. In these cases, the bats were identified as *Pipistrellus pipistrellus sensu lato* by their morphology following Schober and Grimmberger (1997).

c) at foraging sites. I did not base field identification of free-flying pipistrelles entirely on the values of peak frequency of time expanded echolocation calls as this may lead to confusion in regions – such as Italy – where other bat species produce FM-CF calls similar to those of pipistrelles. The Kuhl's pipistrelle *Pipistrellus kuhlii* emits echolocation calls with values of peak frequency which overlap those of *P. pipistrellus* within the range 41.6 - 44.8 kHz (Zingg, 1990), and a similar situation occurs for *P. pygmaeus* and the Schreiber's bat *Miniopterus schreibersii*. This vespertilionid emits FM – CF calls with a highest energy frequency of 48.8 - 70.7 kHz (Zingg, 1990), largely overlapping the range of values known for *P. pygmaeus* (49.2-57.6 kHz; Vaughan *et al.*, 1997b). Barataud (1992) also stressed the difficulty of distinguishing between calls of common pipistrelles and Schreiber's bats.

In order to eliminate all possible risk of incorrect identification, I considered only recordings of bat passes including both FM-CF echolocation calls and the typical social calls emitted by foraging common pipistrelles (Barlow and Jones, 1997b). The Kuhl's pipistrelle emits social calls which are easily separated from those of the common pipistrelle (Russo and Jones, 1999), and *M. schreibersii* does not produce any similar signal. The selected echolocation calls could then be attributed to *P. pipistrellus* when their peak frequency was lower than 49 kHz, and to *P. pygmaeus* for values higher than 52 kHz (Jones and Parijs, 1993). None of these foraging bats called with a peak frequency lying between 49 kHz and 52 kHz. As no description of social calls by Italian common pipistrelles had been given before the present study, I used social calls to identify the bats generically as *P. pipistrellus sensu lato*, but not to tell the two cryptic species apart. In order to avoid data pseudoreplication (Hurlbert, 1984), particular care was taken not to record the same subject more than once.

Recordings were mainly carried out in Campania (southern Italy), Tuscany (north-central Italy) and Sardinia; one pipistrelle was recorded in southern Lazio (Plate 3.2). Tab. 3.1 shows the recording site location, the numbers of bats recorded and the condition under which the recordings were taken (bats leaving roost, foraging, and hand-released). The habitats found at foraging sites and around roosts are also shown.

Recordings were made via the high-frequency output of an S25 bat detector (Ultra Sound Advice, London) connected to a Portable Ultrasound Processor (Ultra Sound Advice, London) which sampled at a rate of 448 kHz and time-expanded (10x) a 2 s sequence of calls. The resulting sequence, lasting 20 s, was then replayed and recorded on Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C. The S25 microphone has a sensitivity of –57dB±3dB (ref. 1V/μbar) from 20-120 kHz.

3.3.2. – Sound analysis

The recordings were analysed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). I used a sampling frequency of 44100 samples/s, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis.

I selected randomly three echolocation calls from each bat, and measured (Plate 3.2) their frequency of highest energy (*FMAXE*), duration and time interval between two consecutive pulses (interpulse interval, *IPI*). A mean value of these parameters was calculated for each bat and used for the analysis.

I analysed one social call from each foraging bat, and measured the following parameters: the number of components to the call (*nocomp*), the total duration of the call (*totdur*), the minimum (*fmin*) and maximum (*fmax*) frequencies of the call (measured on the fundamental), and the mean frequency of highest energy (*freq*) by measuring the peak frequency of each component and calculating the average. For both echolocation and social calls, I measured duration from oscillograms, frequencies of highest energy from power spectra, and took all other parameters from spectrograms.

3.3.3. – Statistical analysis

I applied an Anderson-Darling test to the parameters measured on both echolocation and social calls and found that only some of them conformed to normal distribution. Differences between means of variables which followed a normal distribution were explored with a t-test, while for samples deviating from normality, I carried out univariate analyses by non-parametric tests: differences between medians of each parameter were tested with a two-tailed Mann-Whitney test, and a Spearman's rank coefficient was used to analyse correlation between the peak frequencies of echolocation and social calls. I used a quadratic discriminant analysis with cross validation to distinguish calls of the two cryptic species, and a MANOVA to obtain values for Wilk's λ. This was possible as multivariate techniques are robust to departures from normality (Dillon and Goldstein, 1984). Analyses were carried out with Minitab release 9.2.

3.4. – Results

Forty-two out of 59 bats from peninsular Italy (Campania, Tuscany, Lazio) – mainly recorded during emergence from two roosts in Campania and Tuscany – called with *FMAXE* < 50 kHz.

Only three of them, corresponding to 5.1% of the total, called with a mean *FMAXE* between 49.0-49.9 kHz (Fig. 3.1a). Seventeen called with *FMAXE* > 52 kHz, and were almost all recorded in flight: I found no 'high-frequency' bat roosts, and only three of these subjects were captured in foraging grounds. The frequency distribution of *FMAXE* was clearly bimodal (Fig. 3.1a). Calls fell into two separate ranges, with *FMAXE* averaging 47.4 kHz and 57.3 kHz respectively (Fig. 3.2), and the difference between means was highly significant (Tab. 3.2). Values of *FMAXE* did not overlap between call sets (Fig. 3.1a and Tab. 3.2). An Anderson-Darling test revealed that the frequency distributions of *FMAXE* of 'low-frequency' and 'high-frequency' calls did not deviate significantly from normality ($p = 0.09$ and $p = 0.79$ respectively). The two sets of calls were sufficiently well separated to represent *P. pipistrellus* and *P. pygmaeus* as defined by Jones and Parijs (1993). On average, a difference of 10 kHz occurred between species, and in many cases an experienced listener could tell them apart by hearing the different pitch of time-expanded calls in the field.

Recordings made at the two peninsular roosts (Campania and Tuscany) showed that in both cases only *P. pipistrellus* were present, and no calls with FMAXE above 50.0 kHz were emitted during emergence. I found no relevant difference in call duration between species, while IPI was significantly shorter for *P. pygmaeus* (Tab. 3.2).I also analysed echolocation calls emitted on release by 17 bats captured in Sardinia. Only one of them (Fig. 3.1b) called as a typical '55 kHz' pipistrelle (means± S.D. from three calls of this bat are *FMAXE* = 58.2± 0.10 kHz, duration = 4.0± 0.83 ms, *IPI* = 64.7± 3.06 ms). It was mist-netted at a river site where pipistrelles are often caught (M. Mucedda, *pers. com.*)

and where I also recorded foraging bats - probably pipistrelles judging from their size and flight style – emitting FM-CF echolocation calls which peaked at 54-59 kHz. *FMAXE* of calls by the other Sardinian bats ranged between 46.6 kHz and 52.4 kHz (Tab. 3.2) and showed a unimodal (Fig. 3.1b) normal frequency distribution ($p = 0.08$, n.s., Anderson-Darling test for normality). *FMAXE* of four of these calls' exceeded 49.0 kHz; the highest value (52.4 kHz) was reached by a subject found in the same roost from which other 9 bats examined called with a *FMAXE* of 47.7- 49.5 kHz. Sardinian bat calls showed significantly higher *FMAXE* and shorter duration and *IPI* than peninsular ones (Tab. 3.2).

A sample of 12 social calls from bats emitting echolocation calls with *FMAXE* < 49 kHz, and 14 from those calling with *FMAXE* > 52 kHz, was examined (Fig. 3.3).

Mann-Whitney test and t-test results demonstrated that all parameters measured differed significantly between '45 kHz' and '55 kHz' bats (Tab. 3.3). Social calls of the '45 kHz' bats lasted longer and showed lower values of *fmin*, *fmax*, and *freq* than those of '55 kHz' ones. Furthermore, they mainly consisted of 4 components (range: 3-5), while most of those of '55kHz' pipistrelles showed three components (range: 2-4).

92.3% of the calls were correctly classified to phonic type by a quadratic discriminant analysis with cross-validation and the model was significant (Wilk's $\lambda = 0.13$, $F_{5,20} = 27.8$, $p < 0.001$). No significant correlation was found between *FMAXE* and *freq* within each phonic type (for '45 kHz' and '55 kHz' bats I obtained respectively $r_s = 0.20$, $n = 12$, n.s.; $r_s = -0.18$, $n = 14$, n.s.).

3.5. – Discussion

My results from peninsular Italy provide definite evidence that both cryptic species of pipistrelle bat occur in the country. Not only is this clearly shown by the bimodal frequency distribution of *FMAXE* observed, but it is also confirmed by the significant differences found in social calls between phonic types. At least in Campania, the area I investigated most thoroughly, the two cryptic pipistrelles occur sympatrically, and were in some cases found to share the same foraging habitats (Tab. 3.1). Both species are sympatric in Denmark (H. J. Baagøe, *pers. com.*), Britain (Jones and Parijs, 1993), Northern Ireland (Russ, 1996), Germany (Häussler *et al.*, 1999), Switzerland (Zingg, 1990), France (Lustrat, 1999) and central Spain (Ruedi *et al.*, 1998).

The occurrence of *P. pipistrellus* in central Spain (Ruedi *et al.*, 1998) and southern Italy contradict the general distribution pattern of the two cryptic species in the continent, where *P. pygmaeus* is found round the edge of the pipistrelle range (Mediterrenean basin, Scandinavia), and the intermediate latitudes are occupied by the *P. pipistrellus* (Jones,1997).

The Tuscany roost was a *P. pipistrellus* nursery – on 16th June 1999 I diagnosed late pregnancy by palpation in several females (Racey, 1988) – and two *P. pygmaeus* captured in Campania on 2nd July 1999 were lactating females, their nipples being enlarged and the area around them hairless: therefore, both species seem to breed in the country. *FMAXE* from Italian bats was higher than that observed for the British population (Jones and Parijs, 1993; Vaughan *et al.*, 1997a; Parsons and Jones, 2000) by about 1 kHz and 2-4 kHz for *P. pipistrellus* and *P. pygmaeus* respectively. This difference might be a result of small differences in recording or analysis, or may be related to either geographical variation or habitat structure (Kalko and Schnitzler, 1993; Letard and Tupinier, 1997), as my sample also includes calls recorded in cluttered foraging grounds, which tend to be more frequency modulated (Pye, 1980).

The *IPI* of *P. pygmaeus* was shorter than that of *P. pipistrellus*, as also found by Jones and Parijs (1993), who explained it by considering that bats normally emit one call per wingbeat, and that since *P. pygmaeus* tend to be smaller, and hence produce a higher wingbeat (Carpenter, 1986), a shorter *IPI* is expected for the 'high frequency' pipistrelles.

Data from Sardinia, although from a limited sample, would suggest that both cryptic species occur in the island as well. Further investigations on larger numbers of bats, preferably recorded during roost emergence or foraging in open space, are needed before drawing any conclusion on whether Sardinian pipistrelle calls differ from those made by peninsular populations, as my results would indicate. Such a difference could be the consequence of drift in an isolated population. However, it should be stressed that the whole Sardinian call sample here considered was obtained from hand-released bats. It cannot be ruled out that the differences observed were at least partly due to hand-releasing, as previous work carried out on several bat species showed that under these circumstances bats may emit more frequency modulated calls with *FMAXE* a few kHz higher, and with shorter duration and *IPI* (Parsons and Jones, 2000; D. Russo, *pers. obs.*). The social call differences I found between species are analogous to those described for British bats (Barlow and Jones, 1997b), and show that in the Italy too these signals may be used effectively for telling the two cryptic species apart. My study also confirms that, unlike *P. kuhlii* (Russo and Jones, 1999), *P. pipistrellus* shows no relationship between *FMAXE* of the echolocation calls and *freq*, as also found by Barlow and Jones (1997b).

In order to characterise the Italian population of both cryptic species and to establish their relationship to those of other European countries, future work will have to focus on both morphological and genetical features, and the relative abundance of the two pipistrelles should also be assessed to evaluate their status in Italy.

Tab. 3.1. Location of recording sites in peninsular Italy and Sardinia, numbers of pipistrelles, recording conditions and habitat at foraging sites or around roosts. The ‘N° bats’ column shows the numbers of bats recorded at each site which called with *FMAXE* < 49.0 kHz, between 49.0-51.9 kHz, and ≥ 52.0 kHz, separated by ‘/’. An asterisk in the ‘Situation’ column indicates bats captured at roost; all other hand-released bats were captured at foraging sites.

Site	*N° bats*	*Situation*	*Habitat*
Astroni, Naples Campania: 40°51’N, 14°16’E	0/0/1	Hand-released	Lake, woodland
Alife, Benevento Campania: 41°20’N, 14°18’E	0/0/2	Hand-released	Tree lines bordering arable land
Serino, Avellino Campania: 40°51’N, 14°51’E	1/0/0	Foraging	Woodland managed for chestnut production
Mt. Taburno, Benevento Campania: 41°06’N, 14°37’E	3/0/0	Foraging	*Fagus sylvatica* woodland
Volturno river, Benevento Campania: 41°07’N, 14°46’E	1/0/1	Foraging	River
Roccamonfina, Caserta Campania: 41°17’N, 13°59’E	0/0/4	Foraging	Woodland managed for chestnut production
Giffoni VP, Salerno Campania: 40°48’N, 14°54’E	1/0/3	Foraging	Woodland managed for chestnut production
Vesuvio Nat. Park, Naples Campania: 40°49’N, 14°24’E	2/0/0	Foraging	Gardens, woodland
San Silvestro, Caserta Campania: 41°06’N, 14°19’E	4/0/5 5/1/0 4/0/0	Foraging Leaving roost Hand-released*	*Quercus ilex* woodland
Circeo Nat. Park, Latina Lazio: 41°14’N, 13°05’E	0/0/1	Foraging	Mediterranean macchia
Foreste Casentinesi Nat. Park, Arezzo, Tuscany: 43°46’N, 13°47’E	16/2/0 2/0/0	Leaving roost Hand-released*	*Fagus sylvatica* woodland
Silis river, Sorso, Sassari Sardinia: 40°47’N, 08°34’E	0/0/1	Hand-released	River
Sorso, Sassari Sardinia: 40°47’N, 08°34’E	1/1/0	Hand-released*	Artificial conifer woodland, arable land
Alà dei Sardi, Sassari Sardinia: 40°39’N, 09°19’E	0/1/0	Hand-released	River, *Quercus* woodland
Bortigiadas, Sassari Sardinia: 40°53’N, 09°01’E	7/1/1	Hand-released*	*Quercus* woodland, Mediterranean macchia
Bolòtana, Sassari Sardinia: 40°20’N, 08°57’E	3/1/0	Hand-released	Stream, *Quercus* woodland

Tab. 3.2. Frequency of highest energy (*FMAXE*), duration and interpulse interval (*IPI*) of echolocation calls produced by 42 *P. pipistrellus* (*P.pi.*) and 17 *P. pygmaeus* (*P.py.*) from peninsular Italy, and by 16 pipistrelles (probably all *P. pipistrellus*) recorded in Sardinia. '$T_{45/55}$' column contains the t-test statistic (asterisk) and the Mann-Whitney T statistic (no asterisk) obtained comparing each parameter between the two species from peninsular Italy. '$T_{S/pI}$' column contains the t-test statistic (asterisk) and the Mann-Whitney T statistic (no asterisk) resulting from comparisons between the Sardinian sample and that of 45 kHz bats from peninsular Italy; '*p*' columns show the corresponding levels of significance. Data on the only typical 55 kHz bat recorded in Sardinia are not shown in Table.

		PENINSULAR ITALY							SARDINIA						
Parameter	Species	Mean±S.D.	Range	Median	Q1	Q3	$T_{45/55}$	*p*	Mean±S.D	Range	Median	Q1	Q3	$T_{S/pI}$	*p*
FMAXE (kHz)	*P. pi.*	47.4±1.22	44.6-49.4	47.7	46.5	48.5	17.5*	< 0.001	48.7±1.30	46.6-52.4	48.7	47.9	49.5	3.43*	< 0.01
	P. py.	57.3±2.20	53.3-61.3	57.0	55.8	59.5									
Duration (ms)	*P. pi.*	5.7±1.02	3.5-7.8	5.9	5.0	6.4	452.5	n.s.	3.9±0.48	3.1-4.6	4.0	3.4	4.2	620.0	< 0.001
	P. py.	5.1±1.11	2.4-6.5	5.5	4.7	5.8									
IPI(ms)	*P. pi.*	103.9±24.81	64.4-175.5	95.1	87.5	114.9	527.0	< 0.05	90.7±7.96	77.6-112.2	90.4	85.6	94.1	3.07*	< 0.01
	P. py.	84.8±16.03	50.1-112.0	83.0	74.0	96.1									

Tab. 3.3. Social call parameters from 12 *P. pipistrellus* and 14 *P. pygmaeus*. 'T' column contains the t-test statistic (asterisk) and the Mann-Whitney T statistic (no asterisk) obtained comparing each parameter between species, and 'p' shows the levels of significance.

	Pipistrellus pipistrellus					*Pipistrellus pygmaeus*						
Parameter	Mean± S.D.	Range	Median	Q1	Q3	Mean± S.D.	Range	Median	Q1	Q3	T	*p*
totdur (ms)	28.5±3.96	20.6-34.0	28.6	25.6	32.2	25.8±2.30	23.5-32.8	25.5	24.4	26.7	128.0	< 0.05
fmin (kHz)	15.1±1.23	14.1-17.4	14.9	14.1	15.8	18.3±1.67	14.9-19.8	18.6	17.2	19.8	12.5	< 0.001
fmax(kHz)	31.5±2.96	24.7-34.5	32.9	29.2	33.7	43.1±4.48	30.4-48.3	43.5	41.4	46.3	8.5	< 0.001
freq (kHz)	20.1±1.59	17.0-22.9	20.1	19.3	21.3	22.6±2.04	20.0-26.8	22.3	20.8	24.2	3.41*	< 0.01
nocomp	4.1±0.52	3-5	4	4.0	4.0	3.0±0.39	2-4	3	3.0	3.0	156.5	< 0.001

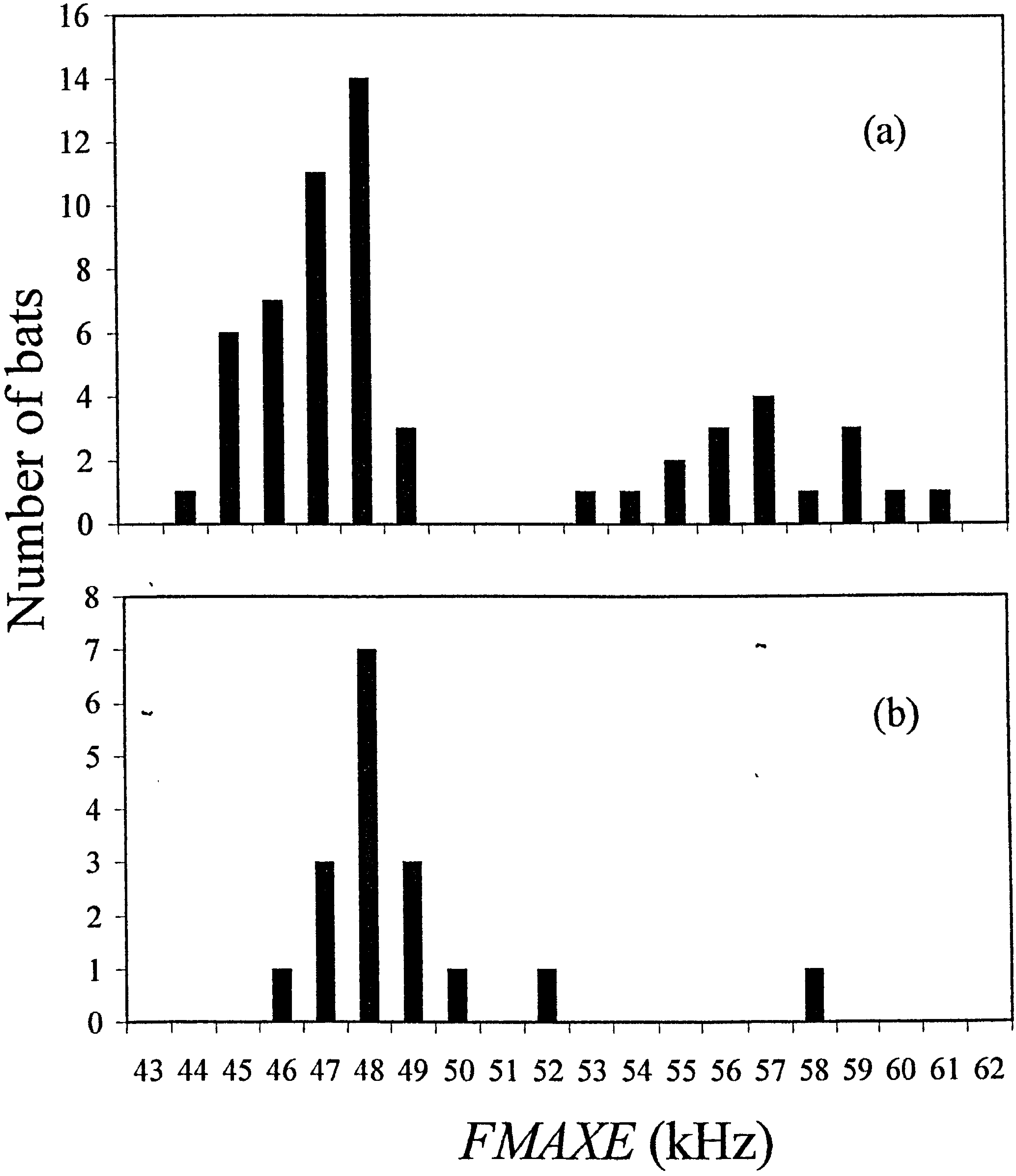

Fig. 3.1. Frequency distribution of search call *FMAXE* by Italian pipistrelles. (a) = peninsular Italy (n = 59); (b) = Sardinia (n = 17). Each class interval contains all frequency values associated with the value listed, e.g. the class labelled "46" includes values between 46.0 - 46.9 kHz.

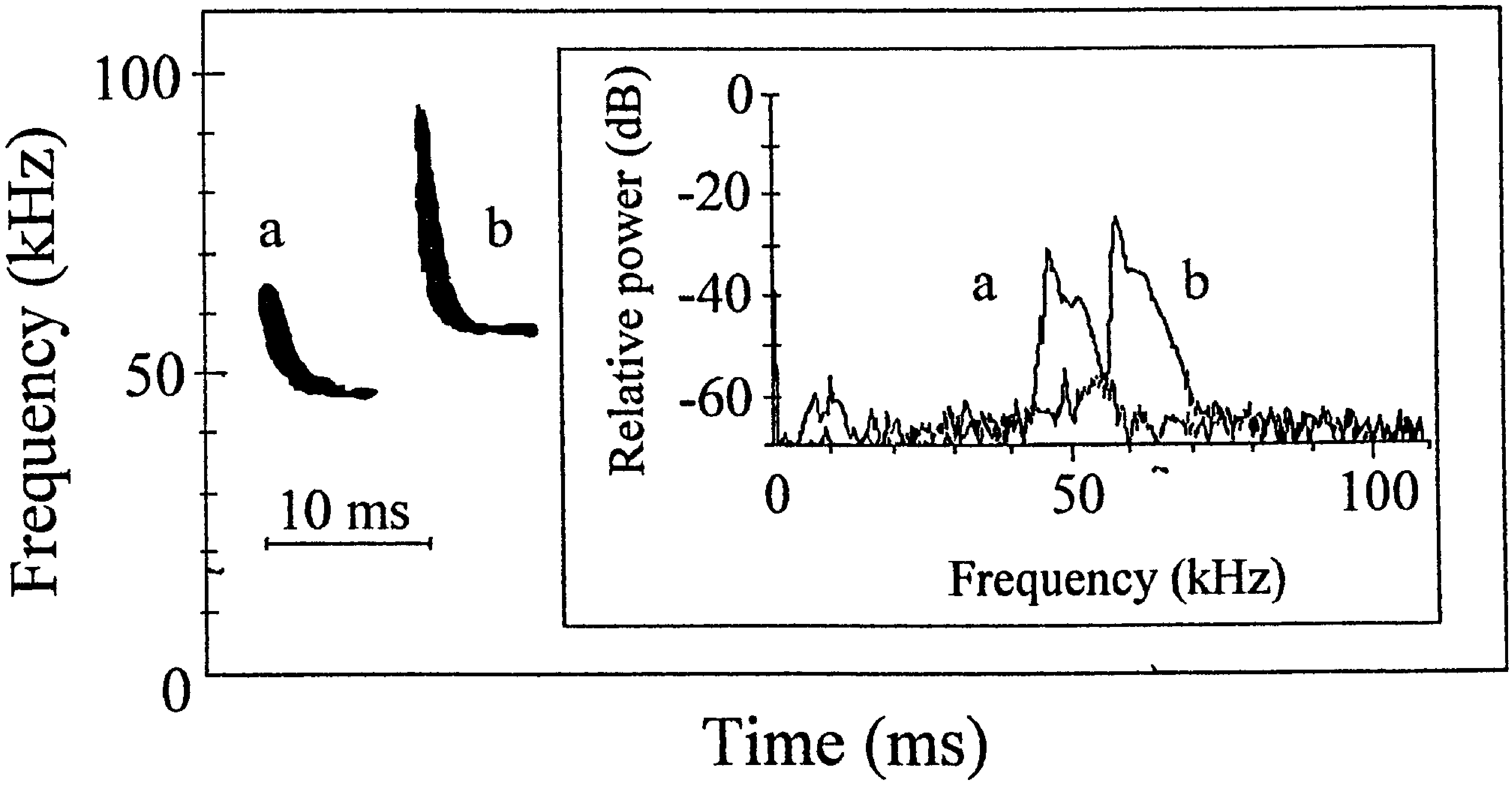

Fig. 3.2. Typical pipistrelle search calls recorded in southern Italy and their overlaid power spectra. (a) = *Pipistrellus pipistrellus*; (b) = *Pipistrellus pygmaeus*. These calls peaked at 47.1 kHz and 57.1 kHz respectively.

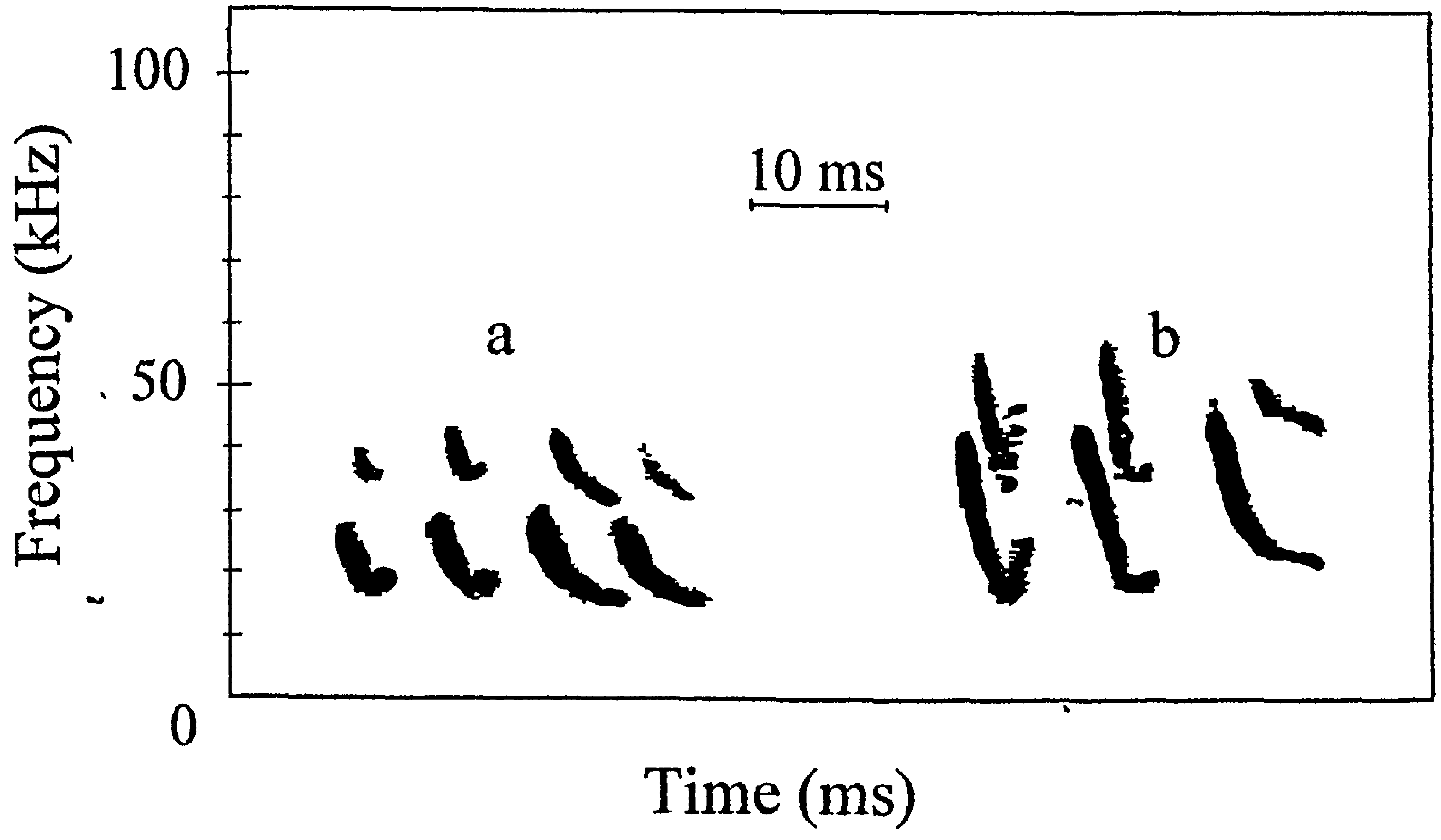

Fig. 3.3. Social calls by Italian pipistrelles. (a) = *Pipistrellus pipistrellus*; (b) = *Pipistrellus pygmaeus*.

Plate 3.1. *Pipistrellus pygmaeus* (above) and *Pipistrellus pipistrellus* (below) from Great Britain (*photograph by G. Jones).*

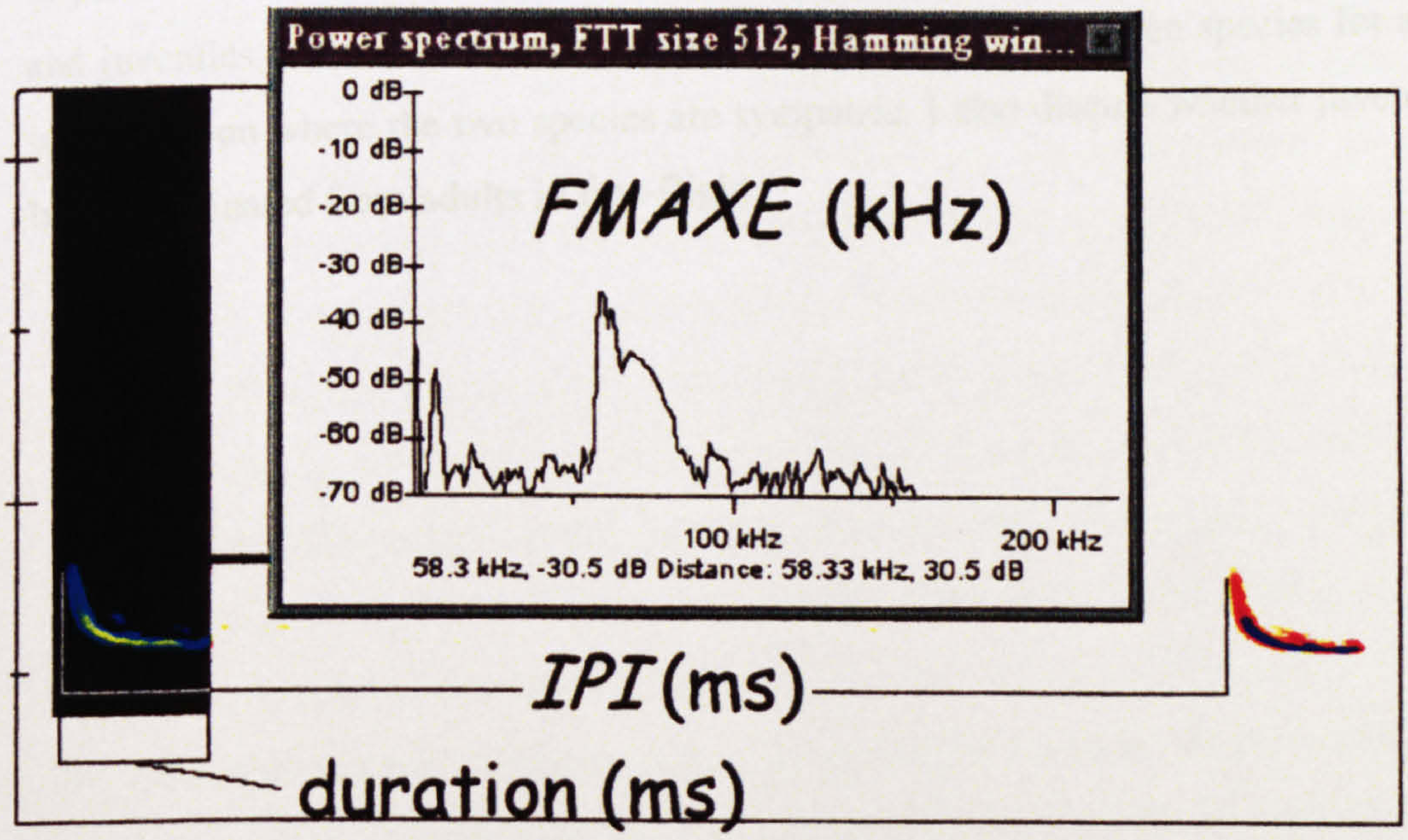

Plate 3.2. Above: regions of Italy where pipistrelles were recorded (a = Campania; b = Lazio; c = Tuscany, d = Sardinia); and a *Pipistrellus pipistrellus/pygmaeus* specimen. Below: a BatSound output (spectrogram, power spectrum) showing measurements taken from pipistrelle echolocation calls. *FMAXE*: frequency of maximum energy; *IPI* = inter-pulse interval. Time measurements are shown on spectrograms for clarity (in this study they were taken from oscillograms).

Four. – Influence of age, sex and body size on echolocation calls of Mediterranean (*Rhinolophus euryale*) and Mehely's (*Rhinolophus mehelyi*) horseshoe bats (Rhinolophidae)

4.1. – Summary

I measured the peak frequency of echolocation calls emitted by hand-held *Rhinolophus euryale* and *R. mehelyi* (resting frequency, RF) respectively from southern Italy and Sardinia and related frequency to sex, age, body size and condition. I analysed echolocation calls from 48 *R. euryale* and 58 *R. mehelyi*. RF of echolocation calls from juveniles was significantly lower than that from adults in both species. In juveniles, RF correlated positively with forearm length, while call frequency showed a positive correlation with body mass in young *R. mehelyi*, but not in young *R. euryale*. I attributed these relationships - absent in adults - to the different growth stage of juveniles. No correlation between body condition index and RF was observed. No significant sexual vocal dimorphism was found in either species whether among adults or juveniles. The age-related difference of RF may facilitate recognition between adults and juveniles. I discuss the implications of overlap in RF between species for acoustic identification where the two species are sympatric. I also discuss whether juveniles can be discriminated from adults in free-flight.

Russo, D., Jones, G. and Mucedda, M. Influence of age, sex and body size on echolocation calls of Mediterranean (*Rhinolophus euryale*) and Mehely's (*Rhinolophus mehelyi*) horseshoe bats (Chiroptera: Rhinolophidae). *Mammalia* 65, in press, is based on this chapter.

4.2. – Introduction

Bats in the family Rhinolophidae emit FM-CF-FM echolocation calls (Schnitzler, 1968), i.e. calls with a long, constant-frequency (CF) component, preceded and followed by two brief, frequency-modulated (FM) components. The second harmonic CF portion contains the maximum energy in the call. By concentrating energy into the second harmonic, rhinolophid ultrasonic calls reach frequencies higher than those heard by tympanate moths. Rhinolophids are therefore able to prey extensively upon these lepidopterans (Jones, 1992). The hearing of rhinolophids show an "acoustic fovea" (Schuller and Pollak, 1979), whereby anatomical and neurological adaptations give an extreme sensitivity to a frequency close to that emitted by a stationary bat (hereafter named "resting frequency", RF). The production of individual-specific frequencies and the existence of a very high capacity of discriminating between slightly different frequencies may potentially allow the caller to signal to conspecifics its identity and other information. When flying, a rhinolophid lowers the peak frequency to compensate for Doppler shifts incurred by moving, so that the frequency of the returning echo will still correspond to that of maximal auditory sensitivity (Schnitzler, 1968).

In rhinolophids and hipposiderids call frequency scales negatively with body size across species (Heller and Helversen, 1989; Francis and Habersetzer, 1998). Within these families, several factors affecting frequency have been identified: colony and geographical location (Heller and Helversen, 1989; Guillén *et al.*, 2000), environmental humidity at hunting grounds (Guillén *et al.*, 2000), body temperature (Huffman and Henson, 1993), size (Jones *et al.*, 1993; Francis and Habersetzer, 1998; Guillén *et al.*, 2000) and body condition (Jones *et al.*, 1994; Guillen *et al.*, 2000), cochlear width (Francis and Habersetzer, 1998), age (Jones *et al.*, 1992; Jones and Ransome, 1993; Jones *et al.*, 1993), and sex (Neuweiler *et al.*, 1987; Jones *et al.*, 1992; Jones *et al.*, 1993; Francis and Habersetzer, 1998).

Bats are also known to communicate by echolocation calls (Fenton, 1985). If call frequency relates to body size, sex and age, then by calling the emitter may signal its physiological and social status (Jones, 1995; Guillén *et al.*, 2000). As higher frequencies make it possible to discriminate smaller targets (Pye, 1993), frequency differences between sexes and between age classes might in theory also constitute mechanisms for intraspecific dietary niche segregation (Jones, 1995), although in practice wavelength differences at very high frequencies are too small to allow substantial differences in target strength between species.

The aim of this study is to investigate the influence of sex, age, body size and condition on echolocation calls in the Mehely's (*Rhinolophus mehelyi*) and the Mediterranean horseshoe bats (*Rhinolophus euryale*) – two species whose echolocation behaviour has not been so fully investigated – from Italian populations (Plate 1.5).

4.3. – Methods

R. euryale was recorded during summers 1998-2000 in Campania and Molise, southern Italy, at three roosts (two nurseries and a non-breeding site). All juveniles of *R. euryale* from the two nurseries were recorded between 22 July and 2 August. Recordings of *R. mehelyi* were carried out on 1 August 1999 at two nursery roosts in Sardinia, the island where the largest Italian populations of this species occur (Lanza, 1959; Mucedda *et al.*, 1994-95). At the time captures were conducted, most juveniles must have been approximately 1.5 months old and were fully capable of flying.

Bats were captured either during the day inside the roost with a hand-net or when leaving the roost by a harp-trap. Body mass and forearm length (FAL) of each bat were measured respectively with a digital balance to the nearest 0.1 g and a caliper to the nearest 0.1 mm. Sex was assessed by inspecting genitalia (Racey, 1988), and wings were trans-illuminated and visually examined to distinguish juveniles from adults, as the former show cartilage epiphyseal plates in finger bones and more tapered finger joints (Anthony, 1988; Plate 4.1). Each subject was then hand-held about 15 cm from the microphone, and its echolocation calls were recorded. As the bat was motionless, calls were not affected by Doppler shift compensation and their frequency corresponded to RF.

Recordings were made via the high-frequency output of an S25 bat detector (Ultra Sound Advice, London) connected to a Portable Ultrasound Processor (Ultra Sound Advice, London) which sampled at a rate of 448 kHz and time-expanded (10x) a 2 s sequence of calls. The resulting sequence, lasting 20 s, was then replayed and recorded on Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C. The S25 microphone has a sensitivity of –57dB±3dB (ref. 1V/μbar) from 20-120 kHz.

Analysis was performed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). I used a sampling frequency of 44.1 kHz, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis. Ten echolocation calls from each bat were randomly chosen; a 15-ms portion from the CF component of each call was selected and RF measured from the power spectrum, and a mean value for the ten calls was then calculated for each subject and considered for analysis. When batches of calls were produced, not more than one call per sequence was analysed.

Preliminary data exploration by Ryan-Joiner and Anderson-Darling tests showed that RF distribution within the whole sample of either species was not normal, and normality was not achieved by data transformation; however, distribution was normal within each age class, with the exception of adult *R. euryale*. Comparisons between age classes were then performed by a Mann-Whitney test, while parametric tests (t-test, ANCOVA) were employed to test for sex differences within age classes (except for adult *R. euryale*). Differences in size between sex and age classes and correlations of RF with body size and with Body Condition Index (BCI = body mass/FAL) were explored with t-tests and correlation analyses respectively. Analyses were carried out with Minitab release 9.2. Means are presented ± SD.

4.4. – Results

I analysed echolocation calls of 106 bats: 32 adult (9 males, 23 females) and 16 young (8 males, 8 females) *R. euryale*, and 28 adult (10 males, 18 females) and 30 young (17 males, 13 females) *R. mehelyi* (Fig. 4.1). RF of echolocation calls from juveniles was significantly lower than that from adults in both species (Figs 4.1 and 4.2, Tab. 4.1). In *R. euryale*, mean RF was 101.40±1.14 kHz and 104.34±0.34 kHz respectively in juveniles and adults. Likewise, in *R. mehelyi* juveniles called on average at 103.68±1.22 kHz, adults at 107.70±0.95 kHz. For both species, a larger variation in call frequency was observed in juveniles (Fig. 4.1, Tab. 4.1). Between species, the RF range of young *R. mehelyi* largely overlapped that of *R. euryale* (Fig. 4.1, Tab. 4.1). In both species, RF of juveniles correlated significantly with FAL, while body mass showed a significant correlation with call frequency in young *R. mehelyi* but not in young *R. euryale* (Tab. 4.2). RF of adults from both species did not correlate significantly with either FAL or body mass (Tab. 4.2). In the field, I noticed a clear relation between growth stage of juveniles and body size, as larger bats (i.e. those with larger FAL and body mass values) showed reduced cartilaginous epiphyseal plates in finger bones. It is therefore the age of young, and not body size, which affects RF. This is further supported by the observation that in adult bats, body size does not influence call frequencies.

An ANCOVA for unbalanced designs (GLM) with FAL entered as a covariate to control for the effect of body size (i.e. that of growth stage) was used in order to test for sexual differences of RF within juveniles. When the influence of FAL was removed, no significant difference between sexes was found for either species (Tab. 4.3). Likewise, no significant frequency difference was found between sexes within adults of either species (Tab. 4.1). Adults showed no marked sexual dimorphism in body size - only differences in body mass of *R. euryale* reached significance between sexes (Tab. 4.1).

No correlation between BCI and RF was found within either adults or juveniles (Tab. 4.2). BCI of adults was significantly larger than that of juveniles in both *R. euryale* (adults = 0.24±0.03; juveniles = 0.19±0.02, t = 5.91, d.f. = 46, $p < 0.0005$) and *R. mehelyi* (adults = 0.26±0.01; juveniles = 0.22±0.01, t = 10.83, d.f. = 55, $p < 0.0005$).

4.5. – Discussion

In both species juveniles called at lower frequencies than adults. Among vespertilionids, this was reported for *Myotis daubentonii* (Jones and Kokurewicz, 1994) and *Myotis lucifugus* (Pearl and Fenton, 1996), and for the rhinolophids *Rhinolophus hipposideros* (Jones *et al.*, 1992) and *Rhinolophus ferrumequinum* (Jones and Ransome, 1993). In the latter species frequency increases during the first 2-3 years of age, then declines – especially after year 10 (Jones and Ransome, 1993). The finding that juveniles (smaller than adults) produce lower calls than adults contradicts expectations that the former, with smaller vocal tracts, will produce higher-pitched calls. Whether such age-related difference has evolved adaptively or represents a mere by-product associated with development of the vocal apparatus is difficult to establish. Producing calls of different frequencies may enable juveniles and adults to recognise each other, and this may play a role in social interactions. My data also illustrate that within juveniles, call frequency differs in relation to the bat's growth stage, i.e. calls might encode information about age and body size of a juvenile. In *R. ferrumequinum*, infant call frequency is, at least in part, learnt from that of the mother (Jones and Ransome, 1993), reinforcing the hypothesis that RF plays an important role in infant-adult recognition and interaction. The possibility that dietary niche segregation occurs between age classes in relation to their RF cannot be confidently supported by the frequency values reported here and in previous studies (Jones *et al.*, 1992; Jones and Ransome, 1993; Jones and Kokurewicz, 1994) because of the limited differences observed between juveniles and adults. In this study, wavelength differences between age classes were of only 0.09 mm and 0.12 mm in *R. euryale* and *R. mehelyi* respectively.

Unlike other rhinolophoid species (Jones *et al.*, 1994; Guillén *et al.*, 2000), in those I studied there was no correlation between RF and BCI. The smaller BCI of juveniles reported might be related to lower foraging success and/or to incomplete body growth. My data do not support any evidence of sexual call dimorphism in *R. euryale* and *R. mehelyi*, agreeing with Heller and Helversen (1989). Among rhinolophids and hipposiderids, lack of call difference between sexes is also known for *R. ferrumequinum* (Jones and Ransome, 1993) and *Hipposideros fulvus* (Jones *et al.*, 1994), while females call at higher frequencies in *Rhinolophus rouxi* (Neuweiler *et al.*, 1987), *R. hipposideros* (Jones *et al.*, 1992), *Rhinolophus creaghi*, *Rhinolophus thomasi* (Francis and Habersetzer, 1998), *Asellia tridens* (Jones *et al.*, 1993), and *Hipposideros ruber* (Guillén *et al.*, 2000). An opposite dimorphism is shown by *Hipposideros speoris*, where males produce higher-pitched calls (Jones *et al.*, 1994).

Although females from the two species I considered here are generally reported to be larger than males, I failed to find significant size differences between sexes, except in the case of *R. euryale* body mass. Further investigations are needed to clarify whether my finding is due to the limited sample analysed or illustrates a real feature of the populations I examined. For *R. euryale*, identical results were obtained from analysis of a larger sample (30 males, 47 females) of adults from the same area (D. Russo, *unpublished data*). The studies so far published show no unequivocal relationship between sexual dimorphism of calls and body size: in fact, call differences between sexes may occur in species where females are larger than males (Jones *et al.*, 1992; Guillén *et al.*, 2000) as well as in those where no sexual dimorphism in body size occurs (Jones *et al.*, 1994; Francis and Habersetzer, 1998; C.M. Francis and A. Guillén, *unpublished data* in Guillén *et al.*, 2000) and, on the other hand, species in which no call difference between sexes occur may show sexual body size dimorphism (Jones and Ransome, 1993; Jones *et al.*, 1994).

The call frequency distribution I obtained for *R. euryale* is wider than that illustrated for Italy by Schnitzler (1968) and is similar to that for southern France (Heller and Helversen, 1989). Doppler compensation is likely to influence ranges of RF to the same extent in both *R. euryale* and *R. mehelyi*, and the degree of frequency overlap between species assessed by comparing resting frequencies is expected to be very similar to that shown by free-flying bats. The overlapping of call frequencies between *R. mehelyi* and *R. euryale* is larger than that previously described (Heller and Helversen, 1989), probably due to the presence of young *R. mehelyi* in the sample whose frequency range extensively overlaps that of adult *R. euryale*. Since I recorded the two species at different geographical locations, I cannot be sure whether the observed frequency overlap also occurs between sympatric populations (in which call frequency might diverge to facilitate intraspecific communication). If overlap occurs, however, a high risk of misclassification would derive from bat detector identification, even when accurate frequency measurements are taken on time-expanded recordings of search calls.

The differences in RF between recently volant juveniles and adults of these species are large enough to allow discrimination between the two age classes once values are corrected for Doppler shift: in this study, all young *R. euryale* called at RF< 103 kHz and adults at values > 103 kHz. *R. mehelyi* showed very little overlap within the range 105.3-105.5 kHz, in most cases juveniles and adults being well separated by their call frequencies. In order to obtain counts of juveniles with no disturbance to colonies, a real-time ultrasound recording device should make it possible to classify each individual emerging from the roost as either adult or juvenile, while an infra-red video-camera could help reduce possible biases deriving from double passes and clustered emergence of bats.

Tab. 4.1. Descriptive statistics for *R. euryale* and *R. mehelyi* RF (of adults and young), FAL and body mass (of adults) and results of relative statistical comparisons. Columns "sex" and "age" show values of Mann-Whitney W (asterisk) and t (no asterisk) statistics for comparisons of variables between sexes (within adults only) and age classes. M = males, F = females. Effects of sex on RF of juveniles (tested with ANCOVA) are reported in Tab. 4.3.

	VARIABLE	SAMPLE AGE	SAMPLE SIZE	MEAN	SD	MIN	MAX	SEX	AGE	*p*
R. euryale										
	RF (kHz)	Adults	M (9)	104.32	0.39	103.64	104.67	383.5(*)		NS
			F (23)	104.35	0.33	103.44	104.73			
		Juveniles	M (8)	100.96	1.24	98.79	102.89			
			F (8)	101.85	0.88	100.32	102.88		1040.0(*)	<0.0005
	FAL (mm)	Adults	M (9)	47.7	1.55	45.2	49.6	0.43		NS
			F (23)	47.9	1.22	45.7	49.7			
	Body mass (g)	Adults	M (9)	10.6	0.86	9.6	12.2	2.27		<0.05
			F (23)	11.8	1.43	10.0	15.4			
R. mehelyi										
	RF (kHz)	Adults	M (10)	108.10	0.88	106.79	108.97	1.73		NS
			F (18)	107.48	0.94	105.31	108.88			
		Juveniles	M (17)	103.80	0.87	102.08	105.53			
			F (13)	103.53	1.59	100.31	105.49		1243.0(*)	<0.0005
	FAL (mm)	Adults	M (10)	51.0	0.79	50.0	52.7	0.45		NS
			F (18)	51.1	0.83	48.6	52.3			
	Body mass (g)	Adults	M (10)	13.1	0.58	11.9	14.1	1.22		NS
			F (18)	13.4	0.62	12.0	14.9			

Tab. 4.2. Correlations of RF with FAL, body mass and BCI in adults and juveniles of *R. euryale* and *R. mehelyi*. DF =degrees of freedom.

	Rhinolophus euryale						*Rhinolophus mehelyi*					
	Adults			Juveniles			Adults			Juveniles		
	r	DF	*p*	R	DF	*p*	r	DF	*p*	r	DF	*p*
FAL (mm)	0.16	30	NS	0.72	14	<0.0005	-0.01	26	NS	0.60	27	<0.0005
Body mass (g)	0.10	30	NS	0.44	14	NS	0.02	26	NS	0.50	27	<0.05
BCI (g/mm)	0.07	32	NS	0.23	14	NS	0.02	26	NS	0.33	27	NS

Tab. 4.3. Results of ANCOVA to test for sexual dimorphism in RF of young *R. euryale* and *R. mehelyi*.

	Rhinolophus euryale				*Rhinolophus mehelyi*			
Source	DF	ADJ MS	F	*p*	DF	ADJ MS	F	*p*
FAL (covariate)	1	7.86	12.27	<0.005	1	16.11	16.33	<0.005
RF	1	0.94	1.46	N.S.	1	1.48	1.50	N.S.
Error	13	0.64			26	0.99		
Total	15				28			

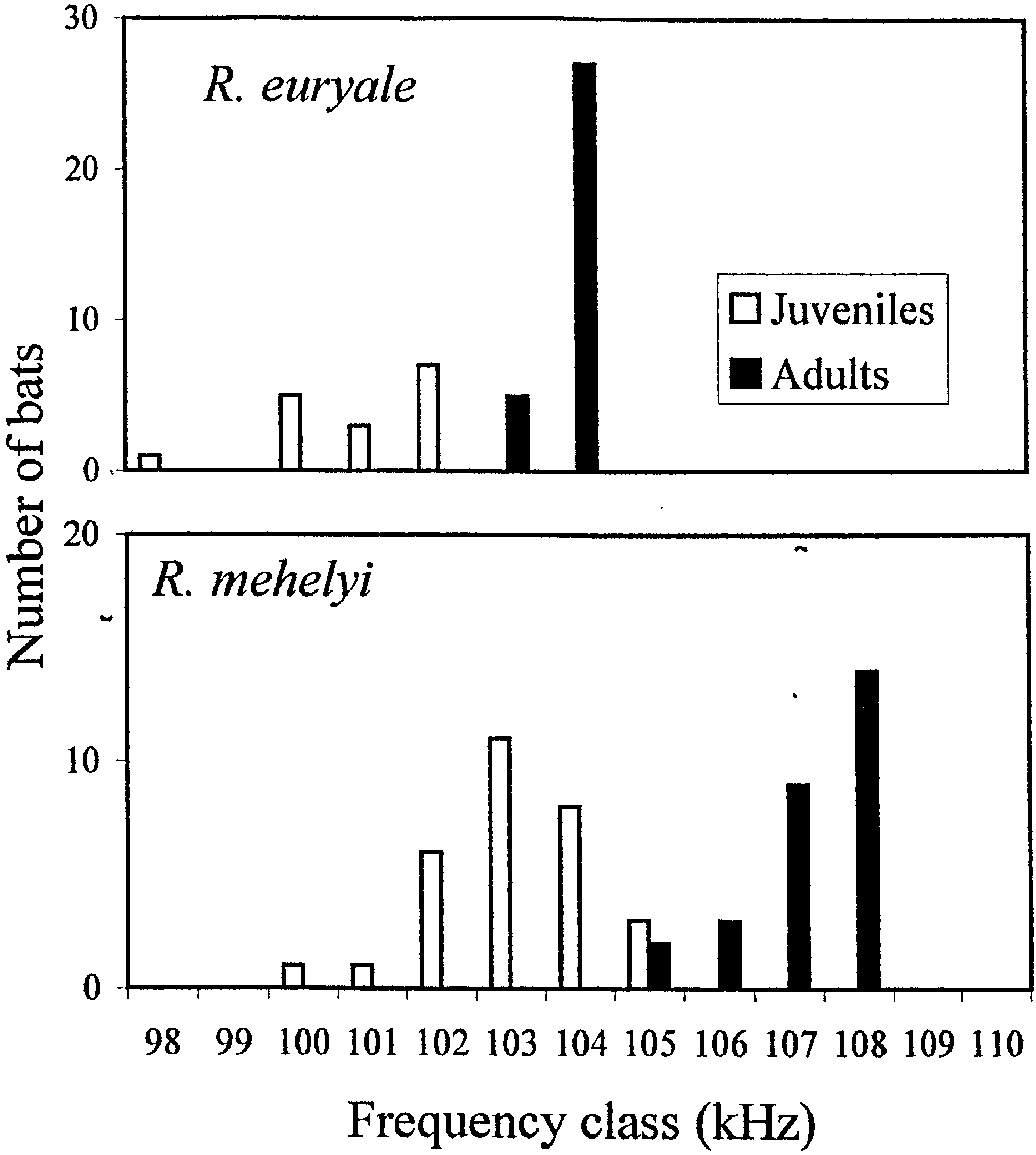

Fig. 4.1. Frequency distribution of RF for 32 adult (9 males, 23 females) and 16 young (8 males, 8 females) *Rhinolophus euryale*, and 28 adult (10 males, 18 females) and 30 young (17 males, 13 females) *Rhinolophus mehelyi*. Each class interval contains all frequency values associated with the value listed, e.g. the class labelled "100" includes values between 100.0 - 100.9 kHz.

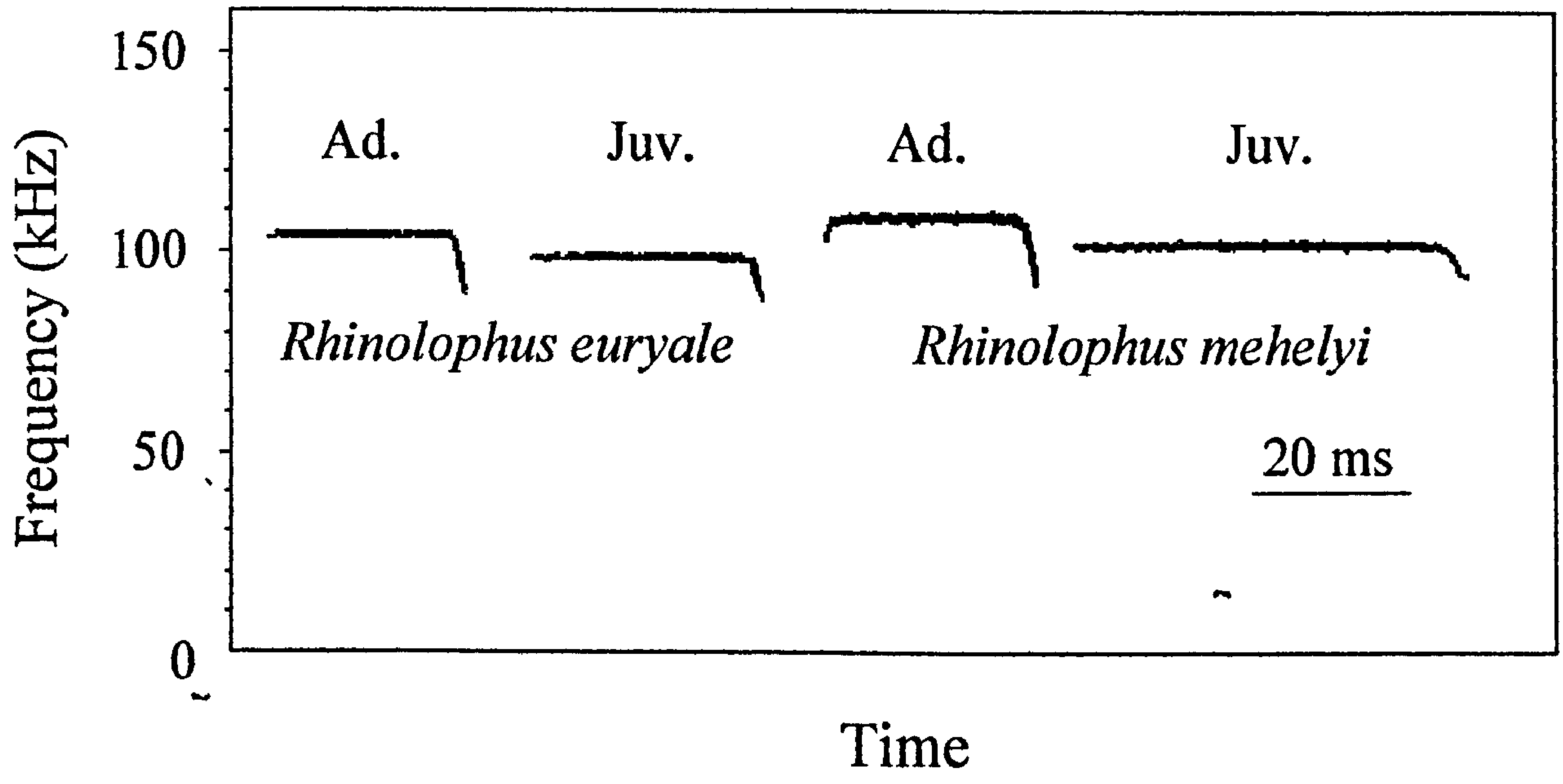

Fig. 4.2. Spectrograms of echolocation calls produced by hand-held *Rhinolophus euryale* and *Rhinolophus mehelyi*. Ad.= Adult; Juv. = Juvenile.

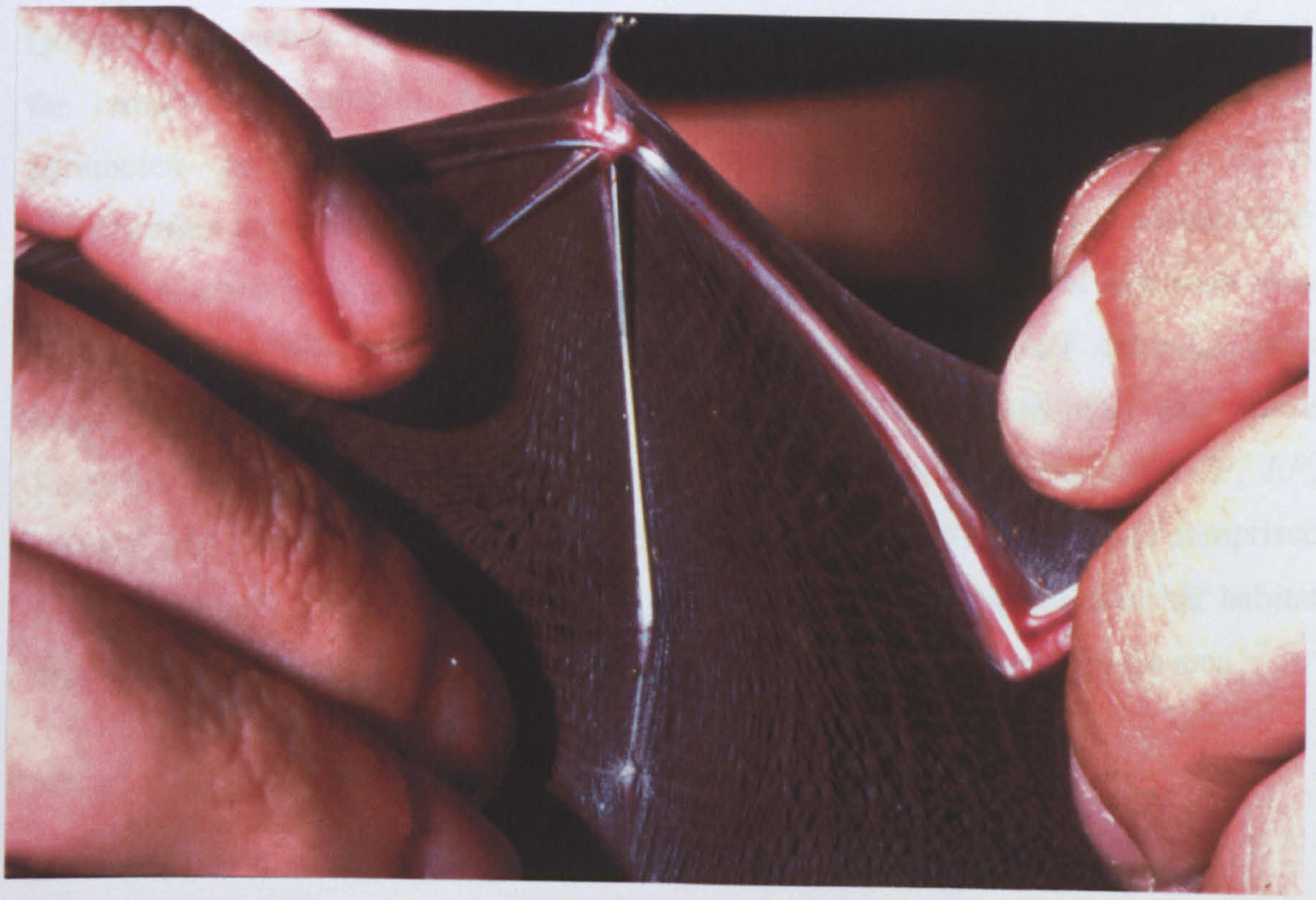

Plate 4.1. Wing of a juvenile *Rhinolophus euryale*. Finger bones show cartilage epiphyseal plates and tapered joints.

Five. – Identification of twenty–two bat species from Italy by analysis of time-expanded recordings of echolocation calls

5.1. – Summary

I describe spectral and temporal features of echolocation calls emitted by 22 bat species from Italy (3 rhinolophids, 18 vespertilionids and the molossid *Tadarida teniotis*). Time-expanded recordings of calls from 950 bats of known identity were examined. *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros*, *Rhinolophus euryale* and *T. teniotis* could be identified by measuring the call frequency of highest energy (*FMAXE*). Quadratic discriminant function analysis with cross–validation was applied to calls from the remaining 18 species. A function based on three spectral and one temporal parameters provided a correct overall classification of approximately 82%. A classification model at genus level that also comprised centre frequency and inter–pulse interval reached 94% correct classification. Two separate discriminant functions were devised for species emitting FM (frequency modulated) and FM–CF calls (i.e. calls consisting of a frequency-modulated component followed by a terminal part whose frequency is almost constant) respectively. The former function included *SF*, *EF*, *FMAXE* and *D* and provided an overall classification rate of 71%; the latter comprised *EF*, *CF*, *D* and *IPI*, and reached 96%. The functions may be applied to bat habitat surveys in southern Italy since they cover most of the species occurring in the area.

Russo, D. and Jones, G. Identification of twenty–two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. *J. Zool., Lond.*, in press, is based on this chapter.

5.2. – Introduction

All 31 European bat species occur in Italy, on the basis of both historical and recent records (Lanza, 1959; Lanza and Finotello, 1985; Lanza and Agnelli, 1999; Russo and Jones, 2000). Bats account for about 30% of the total number of mammalian species in Italy.

In spite of the considerable contribution made by bats to Italian biodiversity and the precarious conservation status of many chiropteran species (e.g. Stebbings, 1988; Mitchell-Jones *et al.*, 1999), little is known about the ecology of Italian bat populations and there is an urgent need for research in this area (Stebbings, 1988).

Suitable foraging grounds are important to bats (e.g. Swift and Racey, 1983; Brigham and Fenton, 1986; Furlonger *et al.*, 1987; Vaughan *et al.*, 1996; 1997a), and the identification of key hunting habitats is therefore an essential step in order to plan bat conservation. Acoustic surveys are an effective way of identifying such habitats, particularly in temperate regions (Kunz and Brock, 1975; Vaughan *et al.*, 1997a).

To identify bat species in flight, Italian researchers have mostly used heterodyne (see Parsons *et al.*, 2000) bat detectors, and more recently time expansion (Pettersson, 1999; Parsons *et al.*, 2000) devices. Their main intent was to map species distribution (e.g. Violani and Zava, 1991, Fornasari *et al.*, 1999). In these studies, however, identification was generally either based on subjective criteria (i.e. it relied on the listener's ability and experience) or carried out by comparing field observations with call descriptions and recordings from other geographical areas (Ahlén, 1981; 1990; Barataud, 1996). In no case was an estimate of the confidence in identification provided.

It is widely documented that echolocation calls may be very similar between species, and that calls show a large within-species plasticity due to geographical location (Thomas *et al.*, 1987), habitat structure, flight height (Miller and Degn, 1981; Zbinden, 1989; Schumm *et al.*, 1991; Obrist, 1995; Jensen and Miller, 1999), and various other physiological and environmental influences (Neuweiler *et al.*, 1987; Heller and Helversen, 1989, Huffman and Henson, 1993; Jones and Ransome, 1993; Jones and Kokurewicz, 1994; Jones *et al.*, 1992; Jones *et al.*, 1993; 1994; Guillén *et al.*, 2000; Russo *et al.*, *in press* b). These factors may have a significant effect on identification. Furthermore, in regions such as Italy where a large number of bat species occur, the use of qualitative criteria in identification brings a higher, uncontrollable risk of misclassification.

Some preliminary attempts were made to devise quantitative identification methods for frequency–divided echolocation calls from Italian bats (Preatoni and Martinoli, 1999). Russo and Jones (1999) showed the diagnostic importance of time-expanded social calls from Italian *Pipistrellus kuhlii*; and Russo and Jones (2000) described time-expanded echolocation and social calls from Italian common pipistrelles, providing evidence that *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* occur in sympatry in the country. Discriminant function analysis (DFA) has been applied successfully to identify bats by their echolocation calls in several areas in Europe (Zingg, 1990; Vaughan *et al.*, 1997b, Parsons and Jones, 2000) and North America (Krusic and Neefus, 1996; Murray *et al.*, 1999). More recently, methods such as synergetic pattern recognition algorithms performing identification in real–time (Obrist *et al.*, *in press*) and artificial neural networks (Parsons, *in press*; Parsons and Jones, 2000) have also been employed.

Models dealing with a large number of species (especially with very similar calls) may show reduced identification performance. Models so far devised for European bats have not included more than 12 species (Zingg, 1990; Vaughan *et al.*, 1997b; Parsons and Jones, 2000).

The aims of my study were: 1) to provide the first comprehensive description of time–expanded echolocation calls from Italian bat populations, 2) to test the performance of a DFA on a larger number of bat species and 3) to devise an objective method of species identification for Italian bats, i.e. a method that is independent from the researcher's subjectivity and ability and that quantifies the degree of certainty of identification. Since most species occurring in peninsular southern Italy were analysed, I also offer a classification method which could be applied to bat habitat surveys within this area.

5.3. – Materials and Methods

5.3.1. – Species recorded and study areas

I recorded 22 bat species. The database of bat calls featured most species occurring in peninsular central and southern Italy. Of the species whose current presence is documented for Italy, my function did not cover: those whose occurrence is limited to the Alps area (*Eptesicus nilssonii, Vespertilio murinus;* Mitchell–Jones *et al.*, 1999); *Rhinolophus mehelyi*, whose populations are mostly confined to Sardinia (Lanza, 1959; Mucedda *et al.*, 1994–95); and *Myotis bechsteinii*, *Nyctalus lasiopterus* and *Pipistrellus nathusii*. Of the last three species, *M. bechsteinii* and *N. lasiopterus* are rare throughout the country, and few confirmed recent records exist (Vergari *et al.*, 1997; Vergari *et al.*, 1998), while *P. nathusii* is uncommon in southern Italy (Lanza, 1959) but was recently documented as breeding in the North (Martinoli *et al.*, 2000). The study area lay between latitudes 44°15'N and 40°09'N. I carried out most field work in southern Italy, i.e. in Campania, Puglia, Abruzzo, Molise and Lazio. Several bat species, however, were exclusively or mainly recorded further north: Tuscany – most of the *Nyctalus leisleri, Plecotus auritus, Pecotus. austriacus* – and Emilia-Romagna – all the *Nyctalus noctula* and several specimens of *Myotis daubentonii* and *Myotis emarginatus.* When possible, bats were recorded at different sites so intraspecific geographical and population variability are represented in the data set.

5.3.2. – Recording conditions and equipment

I made recordings under three conditions:

a) during emergence from roosts where bats of known identity occurred. Each site was visited only once to avoid pseudo–replication (Hurlbert, 1984). Calls were recorded away from the roost exit, so the usually broadband calls emitted immediately after emergence were not included in my dataset;

b) when bats were released from the hand after capture. The bats were mist–netted at foraging sites or while leaving the roost or, on a few occasions, captured inside the roost. Some bats were released in clutter, others in the open. As a result, both calls affected by cluttered environments and those typically emitted in the open were represented in the sample. The first calls in a sequence appeared to have been influenced by release because they were generally steeper in spectrograms and shorter than those emitted away from the release point, and thus were not analysed. In all but two cases, I used data collected on a single visit to each roost. The only *Pl. austriacus* roost I knew was visited twice in two consecutive years: I caught 28 bats inside the roost in 1999 and recorded them in the open, then 25 bats in 2000, and recorded them in clutter. The bats were not marked, so I cannot be sure I did not record some bats twice. However, this risk appears small even without considering mortality or migration, because the colony contained over 100 individuals. Likewise, the only large roost of *Myotis myotis/Myotis blythii* I found was visited twice (in June 1998 and September 2000). In this case, however, I used calls of 30 *M. blythii* and 13 *M. myotis* captured in 2000, and those of 15 *M. blythii* and 26 *M. myotis* captured in 1998. Again, the risk of pseudo-replication is very low as the number of bats captured each time was small, the colony contained several hundred individuals, and the visits were two years apart and at different times of the year. Moreover, only five *M. myotis* and four *M. blythii* captured in 1998 showed the same sex, age class and forearm length of bats captured in 2000.

c) at foraging sites. *Tadarida teniotis* is clearly audible to the unaided ear and easily identified (Zbinden and Zingg, 1986). In order to provide a description of echolocation from Italian *T. teniotis* populations, in all cases this species was recorded in free–flight (Tab. 5.1). I recorded each individual at a different site in order to eliminate the risk of pseudo–replication. Several *P. pipistrellus* and *P. kuhlii*, and most of the *P. pygmaeus* were also recorded in flight (Tab. 5.1) and identified by examining the species–specific structure of their social calls (Barlow and Jones, 1997a; 1997b; Russo and Jones, 1999; 2000). In these cases I made recordings at sites well apart and analysed only one call sampled at each site. In this way only one call from each bat was represented in the sample. Finally, nine passes of *Barbastella barbastellus* recorded in free flight were added to the data–set to make this rare species feature sufficiently in the discriminant function analysis. I recorded free–flying barbastelles at a drinking site where captures conducted over several months had shown the species to occur frequently (to my best knowledge, it is the only site where non–hibernating barbastelles have been captured in peninsular Italy) and identified the recordings by recognising the characteristic alternation of the two call types this species sometimes emits (Barataud, 1996; Parsons and Jones, 2000; see also 'Results'). The barbastelle roosts are likely to have been located in the woodland adjacent to the recording site since bats were mostly mist–netted around about emergence time. I randomly selected the call sequences for analysis among those obtained from several hours of recordings conducted on different nights and at different times of the night in order to minimise the risk of pseudo–replication.

Since my main aim was to devise a method for identifying foraging bats, whenever possible I preferred to record bats leaving the roost or in free–flight (identified by examining social calls), as these conditions are closer (or, in the case of free–flying bats, identical) to those of a foraging bat. It was necessary to record bats on hand–release, however, in a number of cases, i.e.: when I only found small colonies, if any, of a particular species; when bats roosted either alone or in very small numbers, making it necessary to capture them to ensure successful recordings (this was the case with most *N. leisleri*, which occupied bat boxes); when the subjects to be recorded roosted together with bats from other species emitting similar calls (e.g. *M. myotis* and *M.*

blythii); and when the species emitted faint calls (*Plecotus* spp.), so that the recording distance had to be reduced.

Recordings were made via the high–frequency output of an S25 bat detector (Ultra Sound Advice, London) connected to a Portable Ultrasound Processor (Ultra Sound Advice, London) which sampled at a rate of 448 kHz and time–expanded (10x) a 2 ms sample of sound. The resulting sample, lasting 20 s, was then replayed and recorded on Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C (Plate 5.1). The S25 microphone has a sensitivity of –57dB±3dB (ref. 1V/μbar) from 20–120 kHz.

5.3.3. – Sound analysis

The recordings were analysed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). I used a sampling frequency of 44.1 kHz, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis. A 112 Hz frequency resolution was obtained for spectrograms and power spectra.

One echolocation call (selected randomly) from each bat was analysed for all species except *B. barbastellus*. For this species, I analysed two calls – one for each call structure – for 5 bats (4 hand–released, 1 in free–flight). Although a larger number of barbastelles alternated calls in flight, I chose only the bats that were best recorded to limit replication of calls from the same individual in the sample.

The following six parameters were measured from each call (Plate 5.2): start frequency (*SF*), end frequency (*EF*), centre frequency (*CF*, i.e. the frequency of highest energy taken at half call duration), frequency of maximum energy (*FMAXE*), duration (*D*) and inter–pulse interval (*IPI*), i.e. the time interval between two consecutive calls.

D and *IPI* (in ms) were measured from oscillograms, *FMAXE* (in kHz) from power spectra, and all other spectral parameters (in kHz) from spectrograms. In *Plecotus* spp., the highest energy may be in either the fundamental or in the second harmonic (e.g. Parsons and Jones, 2000), therefore *FMAXE* was taken from the harmonic with highest energy, while all other measurements were taken from the fundamental. For all other species, measurements were taken from the harmonic containing most energy, i.e. always from the fundamental in all other vespertilionid and *T. teniotis* calls and from the second harmonic in rhinolophid calls.

5.3.4. – Statistical procedures

For each species, descriptive statistics (mean±SD) are shown. Univariate inferential procedures (ANOVA for normally distributed variables, Mann–Whitney and Kruskal–Wallis for those that did not conform to normality) were used to test for differences between species whose calls had been recorded under identical and well controlled conditions.

Multivariate discriminant function analysis (DFA) with cross–validation was applied to call parameters from 18 species. Because several variables departed from univariate normal distribution – a necessary prerequisite for multivariate normality (MacArdle, 1994) – it followed that the data set did not conform to multivariate normal distribution. However, multivariate tests are robust to departures from normality (Dillon and Goldstein, 1984). Box's M test showed that covariance matrices were not homogeneous ($p < 0.001$), and quadratic analyses were therefore used (Dillon and Goldstein, 1994; Vaughan *et al.* 1997b; Parsons and Jones, 2000). Wilk's λ values were obtained with a MANOVA to test for statistical significance of DFA models, and to assess discrimination power of each variable (Parsons and Jones, 2000). Correlation analyses (Spearman's rank coefficients) was used to explore the strength of relationship between model variables. All tests were performed with MINITAB release 9.2 except Box's M test which was performed with SPSS for Windows version 10.

5.4. – Results

5.4.1. – Description of echolocation calls

I recorded echolocation calls from 950 individuals, 46.3% were recorded during roost emergence, 45.8% on hand–release, and 7.9% in free–flight (Tab. 5.1).

5.4.1.1. – Rhinolophids

The three species I examined – *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros*, *Rhinolophus euryale* – all emitted typical FM–CF–FM echolocation calls, i.e. calls with a long constant–frequency (CF) component preceded and followed by a brief, frequency–modulated (FM) sweep (Fig. 5.1). Echolocation calls from *R. ferrumequinum* showed lower values for all frequency parameters, which did not overlap those of *R. hipposideros* and *R. euryale* (Tab. 5.2). As there was no overlap between species, this variable may be used for species identification; therefore, rhinolophids were not included in the discriminant function analysis.

A Kruskal-Wallis test and Dunn's non-parametric *post-hoc* test showed that of the three species, only *R. ferrumequinum* differed in time parameters, as it produced calls with significantly longer *D* ($H = 13.43$, d.f. = 2, $p < 0.005$) and *IPI* ($H = 20.13$, d.f. = 2, $p < 0.001$). Statistical comparisons were possible as most rhinolophid calls were recorded on roost emergence (Tab. 5.1) in similarly cluttered situations.

5.4.1.2. – Genera Myotis, Plecotus, Barbastella

These species all produced FM calls and could be grouped accordingly (Fig. 5.2, 5.3). *Myotis nattereri* and *M. emarginatus* calls were characterised by the highest mean *SF* (> 105 kHz) of all *Myotis* species; most calls of the former differed from those of the latter in having a clearly lower *EF*, and consequently a larger bandwidth (Tab. 5.2, Fig. 5.2). As one would expect, the two cryptic species *M. myotis* and *M. blythii* emitted similar calls. I obtained comparable sample sizes of both species (Tab.5.1), and since all subjects were recorded under identical and well–controlled conditions (i.e. hand–released in open habitat, mostly from the same roost), it was possible to make a comparison of call parameters. *M. myotis* showed significantly lower values of *FMAXE* (ANOVA, $F_{1,89} = 5.15, p < 0.001$), *EF* (Mann–Whitney $W = 1364.5, p < 0.0001$), and a longer *IPI* ($W = 2247.0$, $p < 0.05$); no difference between species was found for *SF* (ANOVA, $F_{1,89} = 3.10$, NS), *CF* ($W = 2061.5$, NS), and *D* (ANOVA, $F_{1,89} = 1.60$, NS). *Myotis capaccinii* and *Myotis daubentonii* also produced similar calls. A comparison between 16 *M. capaccinii* and 41 *M. daubentonii* – all hand-released – showed significant differences in *EF* (medians were for *M. capaccinii* = 38.0 kHz, *M. daubentonii* = 32.0 kHz, Mann–Whitney $W = 763.0, p < 0.0001$; see also Fig. 5.2) and *D* (means were for *M. capaccinii* = 4.0 ±0.81 ms, and for *M. daubentonii* = 2.9±0.97 ms, ANOVA, $F_{1,55} = 15.48, p < 0.001$). All other parameters did not differ significantly between species (mean *SF M. capaccinii* = 80.1±9.81 kHz, *M. daubentonii* = 77.6±9.27 kHz, ANOVA, $F_{1,55} = 0.77$, NS; median *FMAXE M. capaccinii* = 47.7 kHz, *M. daubentonii* = 46.8 kHz, $W = 550.0$, NS; mean *CF M. capaccinii* = 53.5±2.06 kHz, *M. daubentonii* = 53.3±3.71 kHz, ANOVA, $F_{1,55} = 0.02$, NS; median *IPI M. capaccinii* = 70.1 ms, *M. daubentonii* = 70.8 ms, $W = 463.5$, NS).

Pl. auritus and *Pl. austriacus* both emitted multi–harmonic echolocation calls (Fig. 5.3). I recorded *Pl. auritus* in a range of different conditions (from moderate clutter to fairly open habitat) and *Pl. austriacus* under two well–controlled conditions (high clutter and completely open space). Although the two species samples separated quite well in a multivariate space (see below), the variety of recording conditions adopted made it difficult to control for habitat effects and thus to explore the differences between species of each variable statistically.

B. barbastellus emitted two differently structured echolocation calls, one ('type 1' in Tab. 5.2 (n = 10), Fig. 5.3) of a narrow-band FM sweep, the other characterised by a peculiar convex frequency-time course ('type 2' in Tab. 5.2 (n = 10), Fig. 5.3). Bats either emitted type 1 calls only, or alternated the two call types.

5.4.1.3. – Genera Pipistrellus, Hypsugo, Miniopterus, Eptesicus, Nyctalus, Tadarida

Calls from these genera were characterised by a more or less prominent constant-frequency component, preceded by a frequency modulated one (FM–CF calls, Fig. 5.4, 5.5). The relative importance of each component varied between species, and within species depended on habitat structure (the FM component was more pronounced in clutter, and reduced or sometimes omitted in open habitats). Of the frequency parameters considered, *EF* was the one that overlapped least between *Miniopterus schreibersii, Hypsugo savii* and the three pipistrelle species considered (Tab. 5.2). This variable may therefore help species identification as it is diagnostic in many cases. Echolocation calls from *H. savii* were often characterised by a narrow bandwidth and a longer duration (Tab. 5.2, Fig. 5.4).

Calls from *Eptesicus serotinus* and *N. leisleri* appeared quite similar in spectral and temporal features (Tab. 5.2, Fig. 5.5); however, since all *N. leisleri* were hand-released and most *E. serotinus* were recorded on emergence (Tab. 5.1), differences between species were not analysed statistically. Call parameters of *N. noctula* only occasionally overlapped those of the two above mentioned species. All *N. noctula* I recorded were flying high above ground and regularly alternated two distinct types of calls ('Types 1 and 2', Tab. 5.2, Fig. 5.5). These were easily recognisable from spectrograms as type 1 showed a sensibly more pronounced FM portion than type 2. Statistical analysis supported this qualitative distinction: 'type 1' calls (n = 17) showed significantly higher values of *SF* (Mann–Whitney test, W = 353.5, $p < 0.0001$), *EF* ($F_{1,40} = 41.27$, $p < 0.001$), *FMAXE* ($F_{1,40} = 57.04$, $p < 0.001$), *CF* ($F_{1,40} = 42.74$, $p < 0.001$), shorter *D* (W = 707.5, $p < 0.0001$) and *IPI* (W = 672.0, $p < 0.0001$) than 'type 2' ones (n = 25).

T. teniotis constantly emitted clearly audible echolocation calls (it was the species which called at the lowest *FMAXE*). In hunting grounds, feeding buzzes produced on prey approach were also clearly audible. This species showed the longest IPI too (up to 1 s, Tab. 5.2).

5.4.2. – Discriminant function analysis

In all eighteen species were considered for DFA. Quadratic discriminant analysis was applied to a) the whole species (18) data set; b) calls lumped together according to genera (8 groups); c) the species groups respectively emitting FM (10 species) and FM-CF (8 species) calls, as done by Vaughan *et al.* (1997b).

5.4.2.1. – All species

The best model included *SF*, *EF*, *FMAXE*, and *D* and produced an overall classification rate of 81.8%: 648 out of 792 calls were correctly classified (Tab. 5.3). Random data classification would be 5.6% correct. A MANOVA showed that the model was significant (Wilk's λ = 0.00273, $F_{68,3027}$ =155.911, $p < 0.001$) and that 77.5% of the variation was explained by the first discriminant function. The first three discriminant functions explained 98.9% of the total variation. Classification rates ranged from 38% (for *Myotis mystacinus*) to 98% (for *P. pipistrellus*). Classification rates > 70% were reached for 12 out of 18 species.

Wilk's λ values illustrated the following decreasing discrimination power for the 6 variables: *EF>CF>FMAXE>D>SF>IPI* (Tab. 5.7). The removal of *CF* increased the DFA performance probably because it minimised correlation between variables. *CF* was in fact highly positively correlated with *SF* and *FMAXE* (Spearman's rank coefficient r_s= +0.9; Tab. 5.8). The model also excluded *IPI*, which showed the lowest discrimination power.

5.4.2.2. – Genus discrimination

The best model for genus identification relied on all 6 variables and reached an overall correct classification of 94.1%: 745 out of 792 calls were correctly classified (Tab. 5.4). Random data classification would be 12.5% correct. A MANOVA showed that the model was significant (Wilk's λ = 0.02474, $F_{42,3657}$=104.542, $p < 0.001$) and that 56.7% of the variation was explained by the first discriminant function. The first three discriminant functions explained 99.8% of the total variation. The model could not be improved further by removing any of the 6 variables. Their discrimination power according to Wilk's λ values (in decreasing order) was: *EF>CF>FMAXE>D>SF>IPI* (Tab. 5.7). Classification rates ranged from 60% (for *Eptesicus*) to 99% (for *Myotis).*

5.4.2.3. – Species from genera Myotis, Plecotus, Barbastella

The best model included *SF*, *EF*, *FMAXE*, and *D* and produced an overall classification rate of 71.3%: 266 out of 373 calls were correctly classified to species (Tab. 5.5). Random data classification would be 10% correct. A MANOVA showed that the model was significant (Wilk's $\lambda = 0.04527$, $F_{36,1350} = 48.181$, $p < 0.001$) and that 82.1% of the variation was explained by the first discriminant function. The first three discriminant functions explained 98.8% of the total variation. Classification rates ranged from 38% (for *M. mystacinus*) to 88% (for *M. capaccinii*). Classification rates > 70% were obtained for 6 out of 10 species. About 20% of *M. blythii* calls were misclassified as *M. myotis*, and about 12% of *M. myotis* signals as *M. blythii.* About 22 % of *Pl. austriacus* calls were attributed to the sibling species *Pl. auritus*. According to Wilk's λ values, the discriminating power of the six variables measured in descending order is as follows: *EF>SF>CF>FMAXE>D>IPI* (Tab. 5.7). Again, the removal of *CF* may have increased the DFA performance because it reduced correlation between variables. *CF* showed a high positive correlation to *SF* ($r_s = +0.9$; Tab. 5.8). The removal of *IPI*, a parameter making little contribution to discrimination, must have improved the model by simplifying it.

5.4.2.4. – Species from genera Pipistrellus, Hypsugo, Miniopterus, Eptesicus, Nyctalus

The best model comprised *EF*, *CF*, *D* and *IPI* and produced an overall classification rate of 95.7%: 401 out of 419 calls were correctly classified (Tab. 5.6). Random data classification would be 12.5% correct. A MANOVA showed that the model was significant (Wilk's $\lambda = 0.00603$, $F_{28,1472} = 164.399$, $p < 0.001$) and that 95.1% of the variation was explained by the first discriminant function. The first three discriminant functions accounted for 99.9% of the total variation. Classification rates ranged from 77% (for *N. leisleri*) to 98% (for *P. kuhlii*, *P. pipistrellus*, *Mi. schreibersii*). Classification rates > 70% were obtained for all 8 species. According to Wilk's λ values, the discriminating power of the six variables measured in descending order is as follows: *EF>FMAXE>CF>D>SF>IPI* (Tab. 5.7). Despite the high discriminating power of *FMAXE*, its presence in the model degraded the DFA performance. This probably happened because of its very strong correlation with *EF* and *CF* (r_s approximated +1;

Tab. 5.8). *SF* had little discrimination power, and was strongly correlated with *EF* and *CF* ($r_s = +0.7$ and $+0.8$ respectively; Tab 5.8).

5.5. – Discussion

The echolocation call parameters in Italian bat populations match those observed in other European areas (Tupinier *et al.*, 1980-81; Ahlén, 1981; 1990; Konstantinov and Makarov, 1981; Vogler and Neuweiler, 1983; Zbinden and Zingg, 1986; Schnitzler *et al.*, 1987; Zingg, 1988; Ahlén, 1990; Zingg, 1990; Heller and Helversen, 1989; Jones and Rayner, 1989; Kalko and Schnitzler, 1989; Zbinden, 1989; Jones and Parijs, 1993; Kalko and Schnitzler, 1993; Schumm *et al.*, 1991; Barataud, 1996; Waters *et al.*, 1995; Vaughan *et al.*, 1997b; Russ, 1999; Parsons and Jones, 2000).

Four species (the 3 rhinolophids and *T. teniotis*) from my study region could be identified with no ambiguity using *FMAXE*. For rhinolophids, this might not always be the case as populations from different geographic areas show large differences in call frequencies (Heller and Helversen, 1989). Heller and Helversen (1989) documented some frequency overlap between *R. hipposideros* and *R. euryale* from Greece. Similarly, Barataud (1996) reported that a 5% overlap may occur between frequency calls of these rhinolophids. Problems in identification may arise in areas where *R. mehelyi* also occurs, as its *FMAXE* may overlap that of *R. euryale* and *R. hipposideros* (Heller and Helversen, 1989; Russo *et al.*, *in press* b). *R. ferrumequinum* showed lower *FMAXE*, longer *D* and *IPI* than the other two rhinolophids: this was predictable since *R. ferrumequinum* is considerably larger in size than the other two species, and larger species tend to produce longer calls, spaced out over longer time intervals, at lower frequencies (e.g. Jones, 1999).

Among calls from *Myotis* spp., those of *M. nattereri* were often identifiable because of their broad bandwidth as also documented by other studies (e.g. Vaughan *et al.*, 1997b; Parsons and Jones, 2000; Siemers and Schnitzler, 2000). Such calls allow the species to detect prey very close to acoustic clutter-producing background (Siemers and Schnitzler, 2000). Echolocation signals from most other *Myotis* species, however, showed similar structures and large overlap in spectral and temporal parameters (e.g. Krusic and Neefus, 1996; Vaughan *et al.*, 1997b) – probably, as Parsons and Jones (2000) pointed out, due to the close phylogenetic relatedness existing among such species. Significant yet slight differences were found between *M. myotis* and *M. blythii*, despite the close phylogenetic ties of these cryptic species (Arlettaz, 1995). Such differences are probably related to the ecological segregation and slight morphological differences occurring between the cryptic species (Arlettaz, 1995; Arlettaz *et al.*, 1997). Since *M. myotis* is on average slightly larger than *M. blythii* (e.g. Schober and Grimmberger, 1997), one might expect it to call at lower frequencies (Heller and Helversen, 1989; Barclay and Brigham, 1991; Jones, 1999), as my study verified. I also observed a significant difference in *EF* between *M. daubentonii* and *M. capaccinii*, as reported – but neither quantified nor explored statistically – by Barataud (1996).

In general, species that emit FM-CF calls were easier to tell apart than those producing FM calls, as also verified by other studies (Zingg, 1990; Vaughan *et al.*, 1997b; Parsons and Jones, 2000). In many cases this discrimination could even be accomplished by measuring only *EF* or *FMAXE*, on account of the generally limited range overlap of these variables. This was not always the case, however: in particular, echolocation calls of *N. leisleri* and *E. serotinus* often showed similar values of the variables in question. All calls of *N. leisleri* had to be obtained from hand-released bats: hence, they were often more frequency modulated than calls emitted by the species in open space, and this might have partly increased the degree of overlap with *E. serotinus* calls. However, the similarity of calls of the two species was also stressed by Vaughan *et al.* (1997b), whose discriminant function for FM-CF calls often misclassified their signals.

In this study, DFA provided a high classification rate for most species, despite the large number of species included. Classification success was similar to or higher than that obtained by previous studies where discriminant analysis was applied to a smaller number of European bat species (Zingg, 1990; Vaughan *et al.*, 1997b; Parsons and Jones, 2000). Had it been possible to record more calls for some of the species considered, the overall correct identification rate would perhaps have been higher. Models dealing with groups of species performed better than the model covering all species. Their drawback is that they involve a degree of subjectivity in attributing an unknown call to either the FM or FM-CF group by visual inspection of the spectrogram shape. In my experience, the difference between such groups is normally clear and such a preliminary classification straightforward. Because of this first subjective examination, however, this procedure cannot be used to devise a fully automated identification system – an attractive goal for the future (Jones *et al.*, 2000).

My models classifying to species level included fewer parameters than those considered in previous studies (Zingg, 1990; Vaughan *et al.*, 1997b; Parsons and Jones, 2000). Highest classification rates were achieved by models that best balanced between the inclusion of variables with a high discriminating power with the removal of highly correlated ones. Strong correlations are common between echolocation call spectral features (S. Parsons, *pers. comm.*). As I verified, the inclusion of a variable that highly correlates with others in a DFA model may degrade classification performance as the parameter will add noise rather than increase discrimination power, already provided by its covariate. As in other studies (e.g. Vaughan *et al.*, 1997b; Parsons and Jones, 2000), the species that emitted FM calls were more frequently misclassified than those producing FM–CF calls, and the lowest classification rates occurred for *N. leisleri* and *M. mystacinus*. Low classification rates for British *M. mystacinus* were obtained with both DFA and neural networks (Vaughan *et al.*, 1997b, Parsons and Jones, 2000).

I was pleased with the high degree of discrimination achieved in species identification given the large number of species involved. Moreover, the inclusion of calls recorded in cluttered situations makes my models conservative, and even higher levels of discrimination may be possible for bats foraging in open habitats. I am aware that devising an identification system based on either DFA or other methods (Obrist *et al.*, *in press*, Parsons and Jones, 2000) for a certain geographical area requires considerable effort. Many bat researchers will find it consuming in terms of time and resources, and will probably continue to rely on less sophisticated methods of acoustic identification. In such cases, however, researchers should adopt extremely conservative criteria, given the well-known high variability of echolocation calls that my study also confirmed. Identification should be limited to a restricted number of species, minimising the risk of misclassification. In all cases, identification criteria should rely on accurate measurements of diagnostic features, defined on the basis of a thorough knowledge of geographical and habitat variability of echolocation calls. Moreover, such criteria should always be clearly stated – see e.g. McAney and Fairley (1988) for *R. hipposideros*, Russo and Jones (1999) for *P. kuhlii*, and Waters *et al.* (1999) for *N. leisleri*. The use of social calls should be used as an aid to identification whenever these are proved to be diagnostic, as in the case of European *Pipistrellus* species (Barlow and Jones, 1996; Barlow and Jones, 1997a; 1997b; Russ, 1999; Russo and Jones, 1999; 2000; Jones *et al.*, 2000).

Tab. 5.1. Species recorded, numbers of recording sites and number of bats recorded. The numbers of bats recorded in each situation are also shown.

Species	N sites	N bats	Situation		
			Hand-released	*Leaving roost*	*Free flight*
Rhinolophus ferrumequinum	6	63	13	50	–
Rhinolophus hipposideros	3	34	5	29	–
Rhinolophus euryale	3	45	8	37	–
Myotis daubentonii	6	55	41	14	–
Myotis capaccinii	3	49	16	33	–
Myotis mystacinus	3	13	13	–	–
Myotis emarginatus	6	52	33	19	–
Myotis nattereri	3	12	12	–	–
Myotis myotis	3	42	42	–	–
Myotis blythii	2	49	49	–	–
Nyctalus noctula	1	42	3	39	–
Nyctalus leisleri	2	13	11	2	–
Eptesicus serotinus	3	15	3	12	–
Pipistrellus pipistrellus	9	61	9	40	12
Pipistrellus pygmaeus	8	27	5	–	22
Pipistrellus kuhlii	7	107	38	58	11
Hypsugo savii	7	37	12	25	–
Plecotus auritus	2	26	26	–	–
Plecotus austriacus	2	55	55	–	–
Barbastella barbastellus	1	15	6	–	9
Miniopterus schreibersii	4	117	35	82	–
Tadarida teniotis	21	21	–	–	21
Total		950	435	440	75

Tab. 5.2. Descriptive statistics for echolocation calls from 22 species of Italian bats - 950 subjects, 1 call/bat except in the case of *Barbastella barbastellus*. For 5 subjects of this species two calls/bat (one for each call type) were analysed. *SF* = start frequency, *EF* = end frequency, *FMAXE* = frequency of maximum energy, *CF* = centre frequency, *D* = duration, *IPI* = inter-pulse interval. Means±SDs are shown above range.

Species	Call structure	*SF* (kHz)	*EF* (kHz)	*FMAXE* (kHz)	*CF* (kHz)	*D* (ms)	*IPI* (ms)
Rhinolophus ferrumequinum	FM-CF-FM	70.2±2.76 62.2-78.5	67.3±4.35 58.1-80.9	81.3±1.27 77.8-83.8	81.3±1.49 78.5-84.2	50.5±16.14 16.3-73.8	90.2±34.12 24.9-186.8
Rhinolophus hipposideros	FM-CF-FM	99.0±3.53 92.3-107.8	96.6±6.61 83.4-110.3	111.1±1.73 107.3-114.0	111.0±1.99 107.2-114.3	43.6±13.01 11.9-61.4	70.4±24.49 14.1-113.7
Rhinolophus euryale	FM-CF-FM	93.8±4.36 87.4-102.9	89.1±2.98 84.2-100.5	102.4±0.91 100.2-104.0	102.4±0.85 100.4-103.8	40.6±17.8 11.9-81.1	75.1±28.20 17.0-162.0
Myotis daubentonii	FM	77.0±9.37 54.7-93.6	32.2±2.00 29.5-39.4	47.0±2.58 41.8-56.5	53.1±3.70 44.9-62.8	3.2±0.99 1.4-5.4	75.5±29.33 27.5-186.0
Myotis capaccinii	FM	83.6±9.78 57.9-100.9	39.7±2.07 32.7-42.5	50.4±3.15 44.4-60.9	56.1±3.83 48.2-65.6	3.8±0.68 1.8-5.2	63.8±16.32 29.1-101.4
Myotis mystacinus	FM	96.4±15.33 70.1-122.0	32.4±3.63 29.4-43.3	47.5±8.65 39.2-68.5	56.2±9.40 47.3-82.3	4.2±0.94 3.1-6.4	113.0±56.30 66.7-251.5
Myotis emarginatus	FM	109.0±19.13 62.2-158.3	41.2±5.17 25.5-52.4	58.0±7.69 43.4-76.3	68.7±11.12 41.8-94.0	3.6±1.18 1.3-5.8	70.1±23.79 27.1-125.6
Myotis nattereri	FM	111.8±22.02 72.5-136.6	24.4±3.62 15.2-28.7	46.9±8.48 36.0-66.8	64.8±15.00 47.3-94.4	4.7±1.30 1.9-7.1	80.1±40.10 31.6-188.9
Myotis myotis	FM	79.6±12.46 52.2-104.5	27.9±2.66 24.7-37.7	39.1±5.22 31.5-53.9	50.6±7.65 36.8-68.7	4.6±1.11 2.5-7.1	109.6±27.68 46.6-159.1
Myotis blythii	FM	74.4±15.47 46.5-107.4	30.4±2.50 26.2-36.0	41.4±4.37 33.0-52.5	49.1±7.69 35.2-66.0	4.3±1.23 2.0-7.3	94.3±31.36 40.0-170.8
Nyctalus noctula	FM-CF Call type 1	37.9±8.60 23.8-52.2	23.7±1.49 21.4-26.2	24.5±1.35 22.4-27.0	24.7±1.76 21.4-28.7	14.7±4.26 8.8-23.4	216.9±99.90 120.3-413.1
Nyctalus noctula	FM-CF Call type 2	23.2±3.39 18.2-30.4	20.1±1.99 17.3-23.0	20.7±1.74 17.5-23.6	21.0±1.89 17.4-24.6	22.1±4.11 13.2-29.9	372.5±144.50 120.2-807.5
Nyctalus leisleri	FM-CF	55.0±8.09 38.6-70.3	27.7±1.40 25.5-29.6	30.7±2.29 27.5-34.9	33.9 ±3.64 28.7-40.2	5.3±1.83 2.9-9.0	187.5±79.80 87.1-378.0
Eptesicus serotinus	FM-CF	50.4±5.54 40.2-59.7	27.1±2.38 22.3-32.0	29.9±4.04 24.6-40.2	31.9±3.66 25.2-36.9	7.3±2.18 2.5-10.8	125.8±15.10 100.3-155.6
Pipistrellus pipistrellus	FM-CF	68.8±10.59 50.8-95.2	46.6±1.72 43.3-49	46.9±1.81 42.6-50.6	47.3±1.95 42.5-52.4	5.9±1.24 3.2-8.6	102.5±33.00 59.9-211.0
Pipistrellus pygmaeus	FM-CF	79.6±13.47 63.8-108.6	56.8±1.92 53.2-60.6	57.7±2.35 53.2-63.2	58.1±2.54 54.9-65.4	5.5±1.36 2.1-8.2	89.1±35.36 51.0-217.1
Pipistrellus kuhlii	FM-CF	72.0±12.84 41.8-97.2	39.6±1.71 35.3-45.2	41.4±1.85 36.7-46.2	43.6±2.73 38.2-50.0	5.7±1.45 2.9-9.5	109.8±40.43 45.6-284.7
Hypsugo savii	FM-CF	47.3±11.70 32.0-79.8	32.8±1.72 29.5-36.0	34.6±1.83 30.8-37.9	35.1±2.46 30.3-40.0	8.1±3.16 3.8-15.3	170.7±79.00 85.7-433.0
Plecotus auritus	FM	44.7±5.52 28.0-57.8	26.0±1.60 23.1-29.4	33.1±4.94 25.5-42.1	33.0±4.51 24.7-41.8	2.3±0.73 1.2-3.8	76.8±38.71 21.8-172.4
Plecotus austriacus	FM	41.4±2.08 37.6-46.5	23.6±2.87 19.0-30.3	32.6±8.67 26.3-60.5	29.8±2.58 24.7-39.2	3.8±1.36 1.4-7.0	105.0±32.90 35.8-194.0
Barbastella barbastellus	FM Call type 1	39.4±4.69 35.2-49.0	28.0±3.39 23.8-36.8	33.2±4.39 29.2-44.7	34.8±3.29 31.9-42.5	3.4±0.74 2.5-5.1	108.4±67.00 41.8-229.0
Barbastella barbastellus	FM Call type 2	43.9±2.84 36.8-47.3	28.9±1.94 25.4-31.9	38.9±3.27 32.9-41.3	38.8±2.15 33.5-40.9	4.3±1.30 2.0-6.6	72.4±32.00 43.2-144.9
Miniopterus schreibersii	FM-CF	85.2±13.30 59.3-113.5	52.1±1.50 47.5-55.7	54.2±2.51 49.4-62.5	56.5±3.39 50.3-68.7	5.8±2.13 2.0-13.8	85.5±26.21 40.0-209.30
Tadarida teniotis	FM-CF	17.0±4.56 13.3-31.2	12.1±1.19 10.9-15.0	13.0±1.47 11.5-18.1	13.0±1.49 11.7-17.2	16.6±3.52 10.5-25.5	622.1±191.90 176.0-1000.0

Tab. 5.3. Discriminant Function Analysis model for all species. Model relied on four parameters (*SF*, *EF*, *FMAXE*, *D*: abbreviations are as in Tab. 5.2). Overall correct classification rate was 81.8% (n = 792). M. myo. = *M. myotis*, M. bly. = *M. blythii*, M. cap. = *M. capaccinii*, M. ema. = *M. emarginatus*, M. dau. = *M. daubentonii*, M. nat. = *M. nattereri*, M. myst. = *M. mystacinus*, P. aus. = *P. austriacus*, P. aur. = *P. auritus*, B. bar. = *B. barbastellus*, P. kuh. = *P. kuhlii*, P. pip. = *P. pipistrellus*, P. pyg. = *P. pygmaeus*, H. sav. = *H. savii*, E. ser. = *E. serotinus*, N. lei. = *N. leisleri*, N. noc. = *N. noctula*, M. schr. = *M. schreibersii*.

	True group																	
Classified as	M.myo.	M.bly.	M.cap.	M.ema.	M.dau.	M.nat.	M.mys.	P.aus.	P.aur.	B.bar.	P.kuh.	P.pip.	P.pyg.	H.sav.	E.ser.	N.lei.	N.noc.	M.schr.
M.myo.	27	9	0	0	0	3	1	0	0	0	0	0	0	0	0	1	0	0
M.bly.	5	24	0	0	3	0	3	0	0	0	0	0	0	1	0	0	0	0
M.cap.	1	1	43	12	3	0	0	0	0	0	1	0	0	0	0	0	0	0
M.ema.	0	0	3	34	1	0	1	0	0	0	0	1	0	0	0	0	0	4
M.dau.	0	7	1	0	46	0	2	0	0	0	0	0	0	0	0	0	0	0
M.nat.	2	0	0	3	0	9	1	0	0	0	0	0	0	0	0	0	0	0
M.mys.	3	4	2	3	2	0	5	0	0	1	1	0	0	1	0	0	0	0
P.aus.	0	0	0	0	0	0	0	40	2	0	0	0	0	0	0	1	0	0
P.aur.	2	1	0	0	0	0	0	12	19	2	0	0	0	0	0	0	0	0
B.bar.	0	0	0	0	0	0	0	3	3	16	0	0	0	0	1	0	0	0
P.kuh.	1	0	0	0	0	0	0	0	0	0	103	0	0	0	0	0	0	0
P.pip.	0	0	0	0	0	0	0	0	0	0	2	60	0	0	0	0	0	0
P.pyg.	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	4
H.sav.	0	1	0	0	0	0	0	0	0	0	0	0	0	35	1	0	0	0
E.ser.	1	2	0	0	0	0	0	0	0	1	0	0	0	0	7	3	4	0
N.lei	0	0	0	0	0	0	0	0	2	0	0	0	0	0	5	8	0	0
N.noc.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	38	0
M.sch.	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	109
Total *N*	42	49	49	52	55	12	13	55	26	20	107	61	27	37	15	13	42	117
N correct	27	24	43	34	46	9	5	40	19	16	103	60	25	35	7	8	38	109
% correct	64	49	88	65	84	75	38	73	73	80	96	98	93	95	47	62	90	93

Tab. 5.4. Discriminant Function Analysis model for genus identification. Model relied on 6 parameters (*SF, EF, FMAXE, D, IPI*: abbreviations are as in Tab. 5.2). Overall correct classification rate was 94.1% (n =792).

	True group							
Classified as	*Myotis*	*Plecotus*	*Barbastella*	*Pipistrellus*	*Hypsugo*	*Eptesicus*	*Nyctalus*	*Miniopterus*
Myotis	269	2	2	2	2	1	1	3
Plecotus	0	76	2	0	0	1	1	0
Barbastella	1	3	16	0	0	0	0	0
Pipistrellus	0	0	0	189	3	0	1	5
Hypsugo	0	0	0	1	32	1	1	0
Eptesicus	1	0	0	0	0	9	6	0
Nyctalus	1	0	0	0	0	3	45	0
Miniopterus	0	0	0	3	0	0	0	109
Total *N*	272	81	20	195	37	15	55	117
N correct	269	76	16	189	32	9	45	109
% correct	99	94	80	97	86	60	82	93

Tab. 5.5. Discriminant Function Analysis for species emitting FM calls (genera *Myotis, Plecotus, Barbastella*: names of species are abbreviated as in Tab. 5.3). Model relied on 4 parameters (*SF, EF, FMAXE, D*: abbreviations are as in Tab. 5.2) and provided an overall correct classification rate of 71.3% (n = 373).

	True group									
Classified as	M.myo.	M.bly.	M.cap.	M.ema.	M.dau.	M.nat.	M.mys.	P.aus.	P.aur.	B.bar.
M.myo.	27	10	0	0	0	3	1	0	0	0
M.bly.	6	25	0	0	3	0	3	0	0	0
M.cap.	2	1	43	12	3	0	0	0	0	0
M.ema.	0	0	3	34	1	0	1	0	0	0
M.dau.	0	7	1	0	46	0	2	0	0	0
M.nat.	2	0	0	3	0	9	1	0	0	0
M.mys.	3	4	2	3	2	0	5	0	0	1
P.aus.	0	0	0	0	0	0	0	40	2	0
P.aur.	2	2	0	0	0	0	0	12	20	2
B.bar.	0	0	0	0	0	0	0	3	4	17
Total *N*	42	49	49	52	55	12	13	55	26	20
N correct	27	25	43	34	46	9	5	40	20	17
% correct	64	51	88	65	84	75	38	73	77	85

Tab. 5.6. DFA analysis for species emitting FM-CF calls (genera *Pipistrellus*, *Hypsugo*, *Eptesicus*, *Nyctalus*, *Miniopterus*: names of species are abbreviated as in Tab. 5.3).Model relied on four parameters (*EF*, *CF*, *D*, *IPI*: abbreviations are as in Tab. 5.2) and provided an overall correct classification rate of 95.7 % (n = 419).

	True group							
Classified as	P.kuh.	P.pip.	P.pyg.	H.sav.	E.ser.	N.lei.	N.noc.	M.sch.
P.kuh.	105	1	0	0	0	0	0	0
P.pip.	1	60	0	0	0	0	0	1
P.pyg.	0	0	24	0	0	0	0	1
H.sav.	1	0	0	36	1	0	0	0
E.ser.	0	0	0	1	12	3	3	0
N.lei	0	0	0	0	2	10	0	0
N.noc.	0	0	0	0	0	0	39	0
M.sch.	0	0	3	0	0	0	0	115
Total *N*	107	61	27	37	15	13	42	117
N correct	105	60	24	36	12	10	39	115
% correct	98	98	89	97	80	77	93	98

Tab. 5.7. Wilk's λ values for call parameters. The lower the value for Wilk's λ, the higher is the discrimination power of the variable. For all variables, Wilk's λ values were highly significant (p <0.001).

Variable	All species	Genera	FM species	FM-CF species
SF	0.25774	0.38993	0.22089	0.32020
EF	0.05385	0.22999	0.19384	0.02760
FMAXE	0.16934	0.37040	0.33028	0.04287
CF	0.15899	0.30624	0.23578	0.06322
D	0.24578	0.40725	0.77026	0.27992
IPI	0.45821	0.51717	0.78514	0.46111

Tab. 5.8. Spearman's rank correlation coefficients for parameters used in the 3 Discriminant Function Analysis models to species level. Abbreviations are as in Tab. 5.2.

All species

	SF	*EF*	*FMAXE*	*D*	*IPI*
EF	0.52				
FMAXE	0.72	0.81			
D	-0.19	-0.07	-0.25		
IPI	-0.44	-0.28	-0.51	0.49	
CF	0.88	0.64	0.88	-0.27	-0.50
FM species					
EF	0.61				
FMAXE	0.72	0.83			
D	0.24	-0.13	-0.16		
IPI	-0.26	-0.36	-0.43	0.31	
CF	0.93	0.72	0.82	0.08	-0.32
FM-CF species					
EF	0.72				
FMAXE	0.76	0.98			
D	-0.55	-0.45	-0.50		
IPI	-0.62	-0.63	-0.65	0.49	
CF	0.81	0.97	0.98	-0.55	-0.66

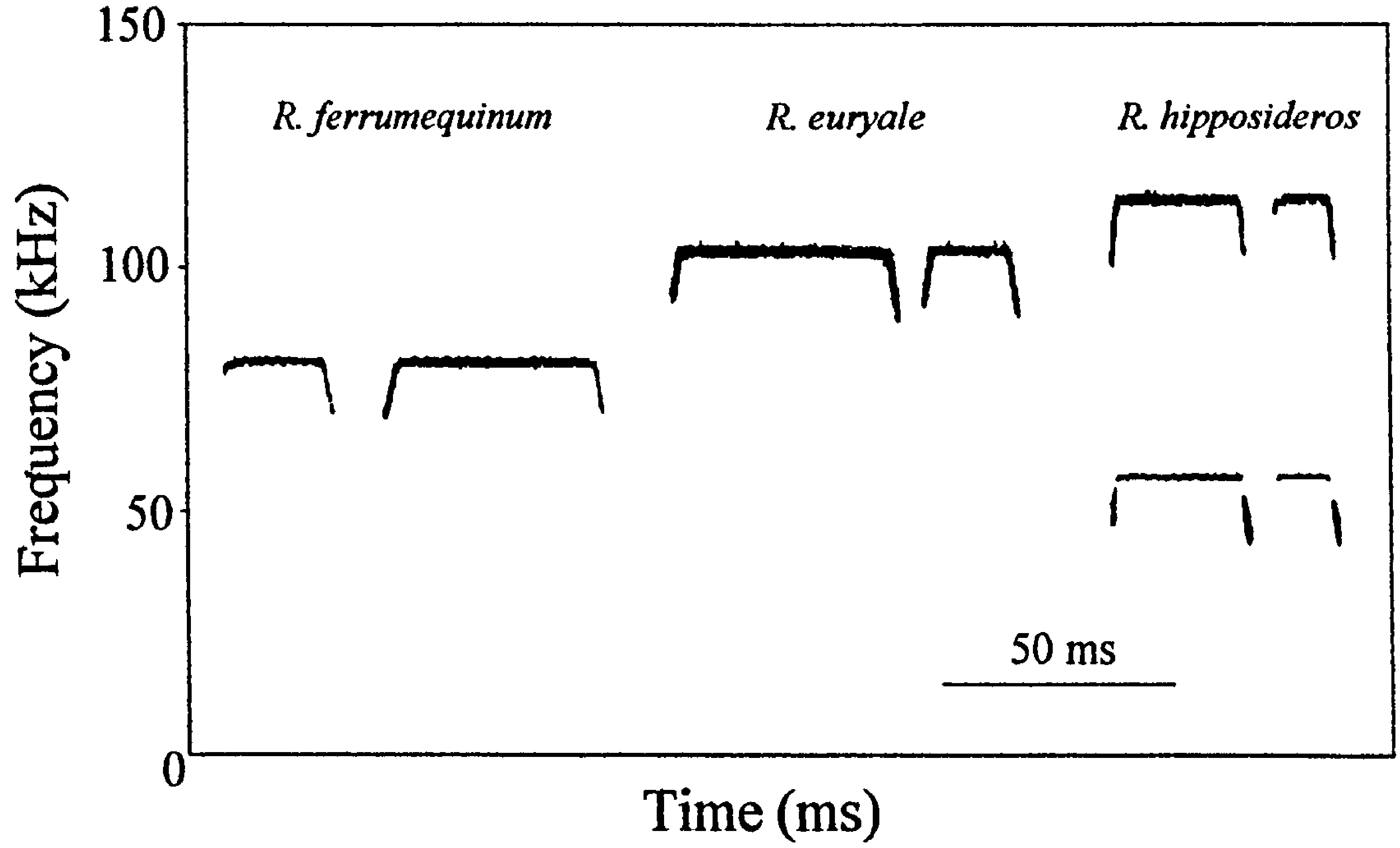

Fig. 5.1. Echolocation calls of *Rhinolophus ferrumequinum* (second harmonic), *Rhinolophus euryale* (second harmonic) and *Rhinolophus hipposideros* (fundamental and second harmonic). Two calls/species are shown to illustrate call variability. Gaps between calls do not represent inter-pulse intervals.

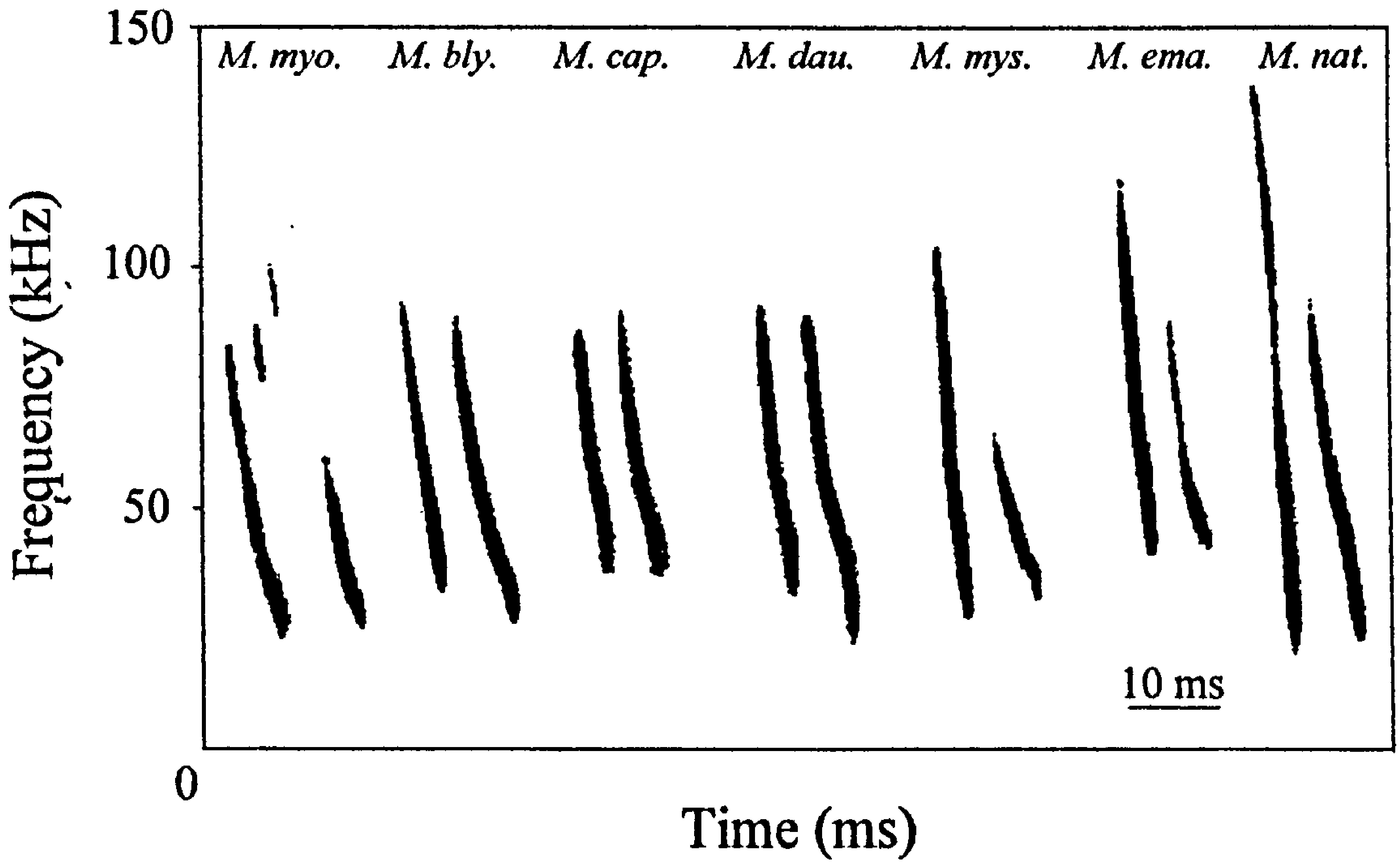

Fig. 5.2. Echolocation calls of *Myotis* species. Two calls/species are shown to illustrate call variability. *M. myo.* = *Myotis myotis*, *M. bly.* = *Myotis blythii*, *M. cap.* = *Myotis capaccinii*, *M. dau.* = *Myotis daubentonii*, *M. mys.* = *Myotis mystacinus*, *M. ema.* = *Myotis emarginatus*, *M. nat.* = *Myotis nattereri*. Gaps between calls do not represent inter-pulse intervals.

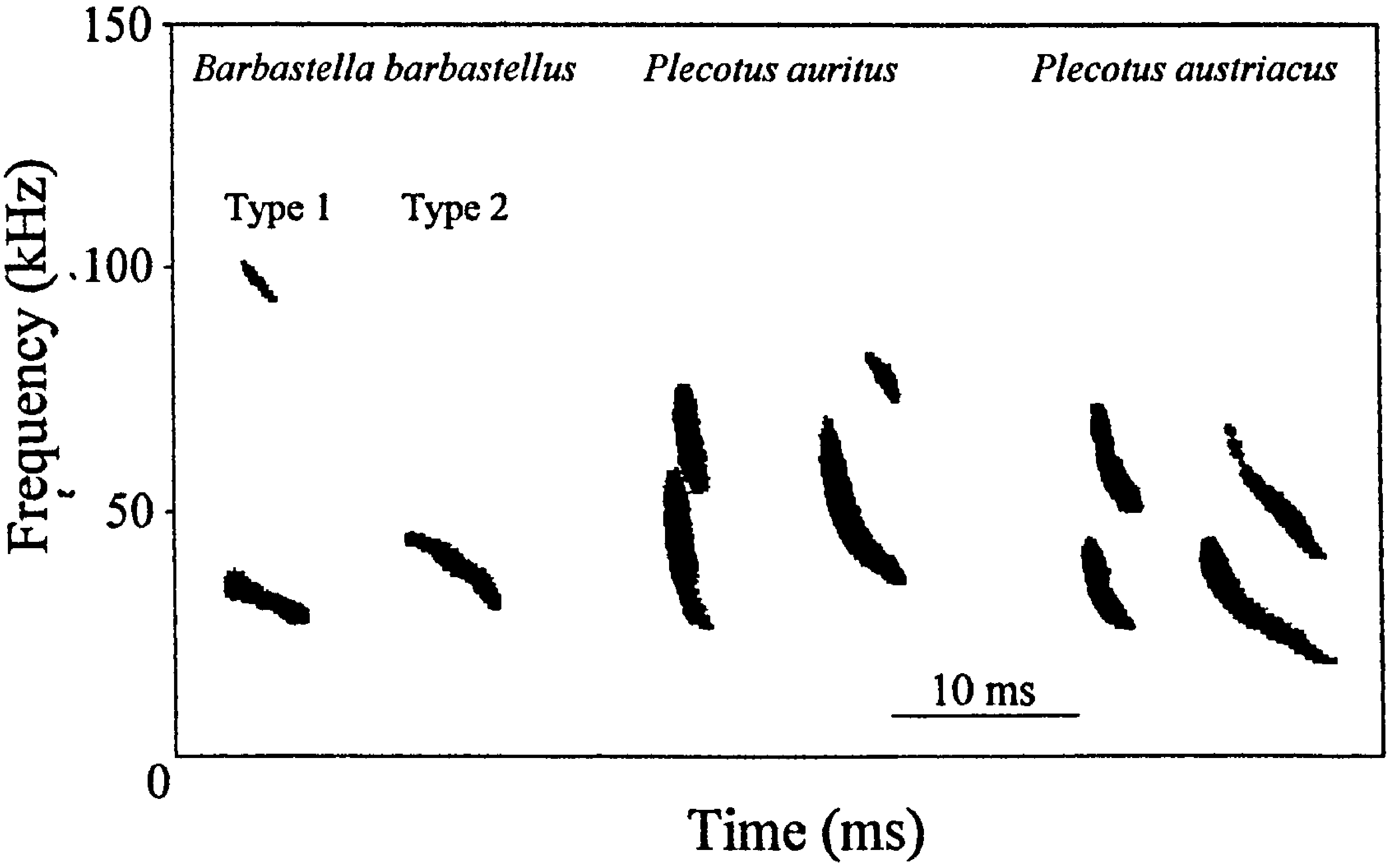

Fig. 5.3. Echolocation calls of *Plecotus auritus*, *Plecotus austriacus*, *Barbastella barbastellus*. Two calls/species are shown to illustrate variability. For each *Plecotus* pair of calls, the first was recorded in clutter and the second in open. The two call types of *B. barbastellus* are also illustrated. Gaps between calls do not represent inter-pulse intervals.

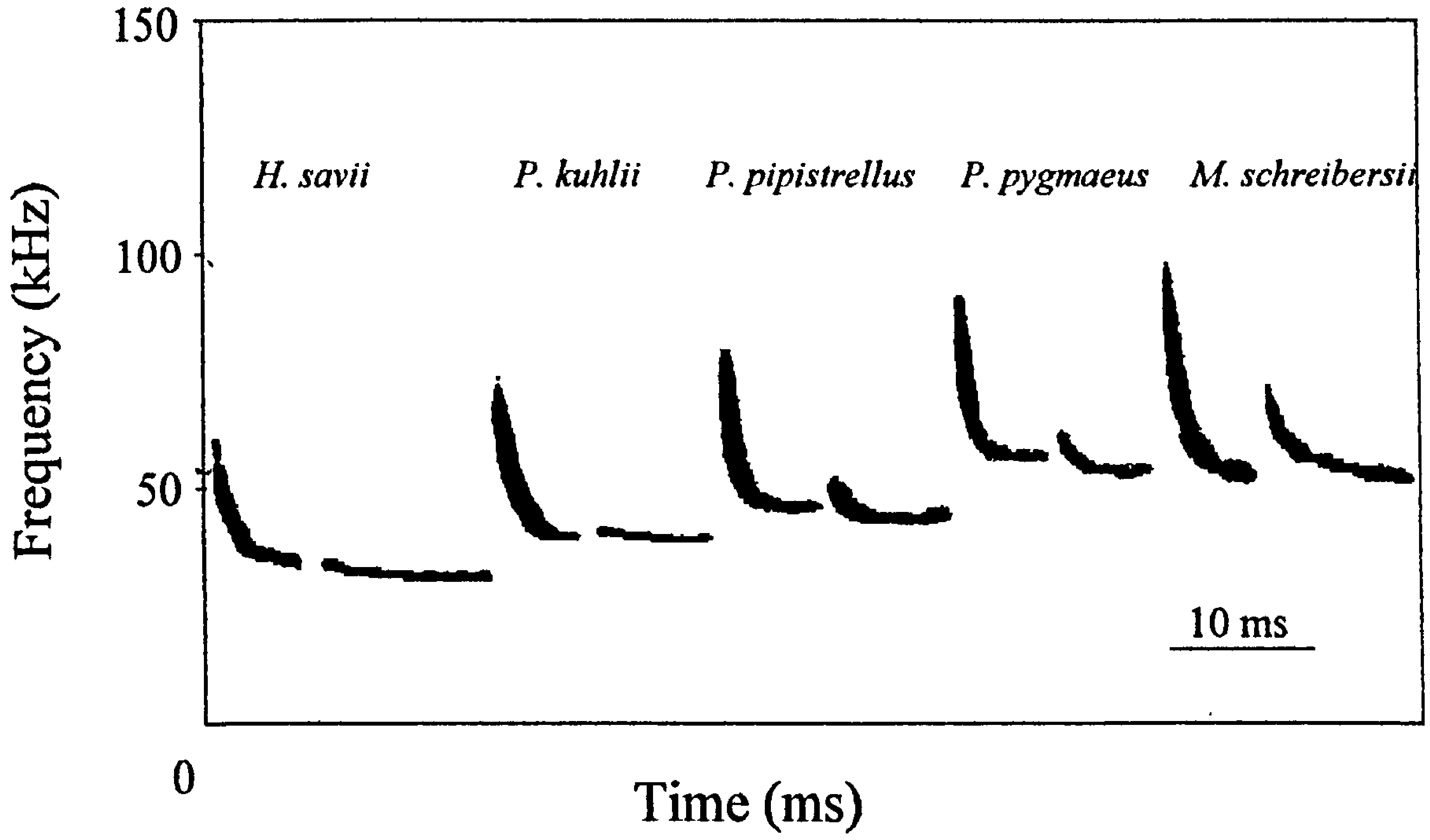

Fig. 5.4. Echolocation calls of *Hypsugo savii*, *Pipistrellus kuhlii*, *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus* and *Miniopterus schreibersii*. Two calls/species are shown to illustrate variability. For each pair of calls, the first was recorded in clutter and the second in open. Gaps between calls do not represent inter-pulse intervals.

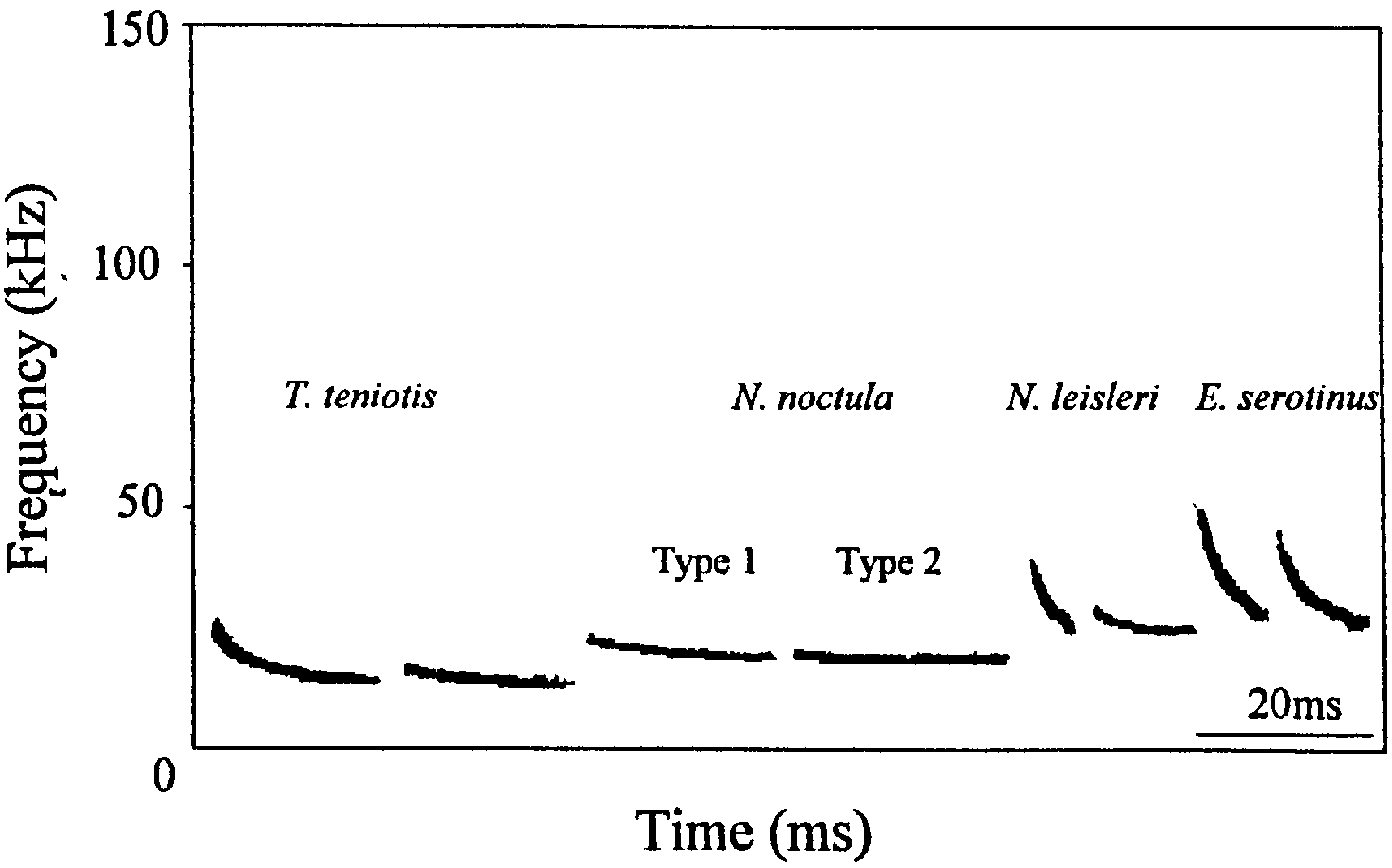

Fig. 5.5. Echolocation calls of *Tadarida teniotis, Nyctalus noctula, Nyctalus leisleri* and *Eptesicus serotinus.* Two calls/species are shown to illustrate variability. For *N. noctula*, both call types are illustrated. For the other species, the first call was recorded in clutter and the second in open. Gaps between calls do not represent inter-pulse intervals.

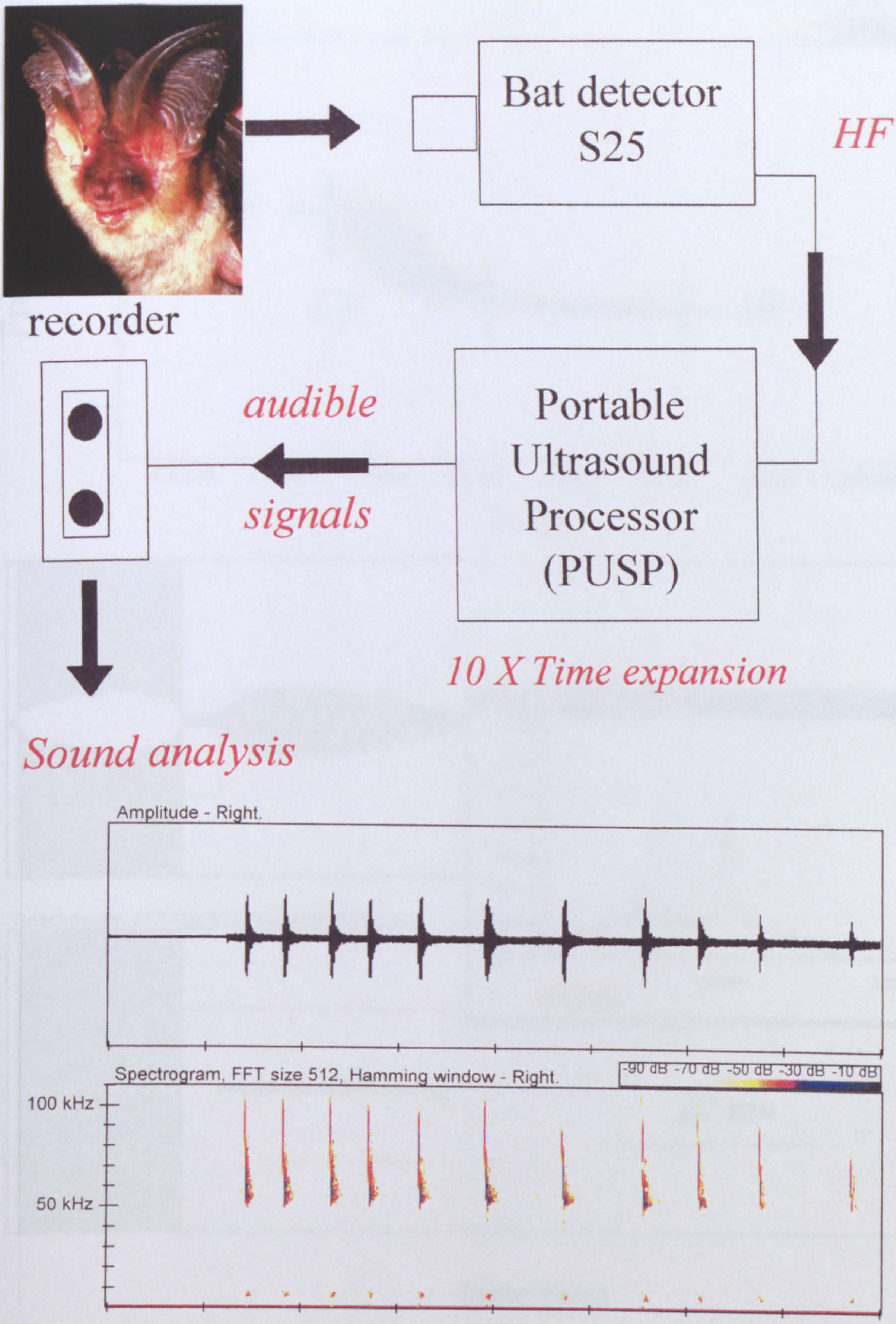

Plate 5.1. The recording procedure adopted for this study. Recordings were taken via the high–frequency output (HF) of an S25 bat detector connected to a PUSP which time–expanded (10x) a 2 ms sample of sound. The resulting sample was recorded by means of a Sony Professional Walkman WM D6C. Analysis was performed with BatSound 1.0: below is a typical software output, oscillogram and spectrogram.

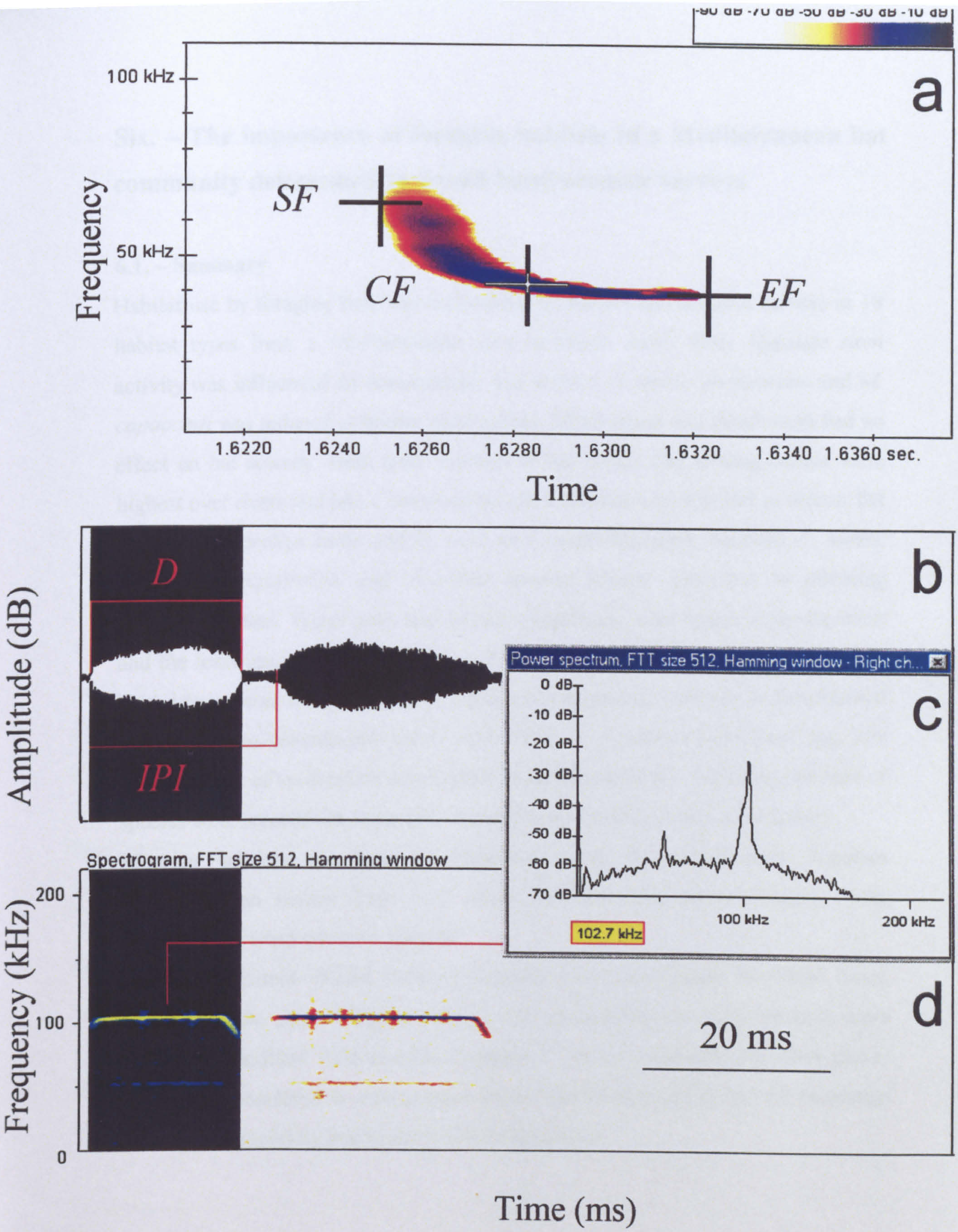

Plate 5.2. Typical BatSound outputs showing the variables measured. a = spectrogram of a pipistrelle call. *SF*, *CF* and *EF*: start, centre and end frequencies. b = oscillograms and d = spectrograms of two *Rhinolophus euryale* calls. *D* = duration; *IPI* = inter-pulse interval. The frequency of maximum energy (102.7 kHz in the example) is taken from the power spectrum (c).

Six. – The importance of foraging habitats in a Mediterranean bat community determined by broad-band acoustic surveys

6.1. – Summary

Habitat use by foraging bats was determined by broad-band acoustic surveys in 10 habitat types from a Mediterranean area (southern Italy). Only *Hypsugo savii* activity was influenced by temperature, and activity of *Myotis daubentonii* and *M. capaccinii* was reduced at higher wind speeds. Moon phase and cloud cover had no effect on bat activity. Both total numbers of bat passes and feeding buzzes were highest over rivers and lakes. Some towns and farmland sites also had an intense bat activity. *Pipistrellus kuhlii* and *H. savii* were most frequently recorded. *P. kuhlii*, *Pipistrellus pipistrellus* and *Tadarida teniotis* proved generalist in choosing foraging habitats. Water sites and conifer plantations were respectively the most and the least used habitats by *H. savii*. Rivers were especially important to *Myotis* bats, *Miniopterus schreibersii* and *Pipistrellus pygmaeus*. Activity in broadleaved woodlands was considerable for *P. pipistrellus*, *P. pygmaeus* and *Myotis* spp. The mean number of species/site was highest in riparian habitats. High total numbers of species were recorded at water sites, broadleaved woodlands and olive groves.

Riparian habitats constitute an important target for conservation. Riparian vegetation can sustain large prey insect densities and shelter foraging spots, especially for wind-sensitive species.

Land management should consider keeping some unmanaged woodland areas, minimising the size of logged patches and maintaining corridors between main blocks of woodland. Traditionally managed *C. sativa* woodlands and olive groves should be considered in conservation plans. Farmland practices should encourage landscape complexity and limit the use of pesticides.

6.2. – Introduction

Transformation of foraging habitats may seriously affect insectivorous bat populations (Stebbings, 1988; de Jong, 1995; Vaughan et al., 1996; Law et al., 1999; Mitchell-Jones et al., 1999; Hutson et al., 2001). Habitat features and quality influence prey insect biomass, diversity and distribution (e.g. Fry and Lonsdale, 1991). It is therefore essential to identify the habitat types and characteristics preferred by bat species in order to define appropriate conservation guidelines and to apply effective protection measures.

The habitat requirements of European bat species may differ according to latitude (Racey, 1998). Although a considerable amount of information on bat habitat use is available for several geographical areas (e.g. Furlonger *et al.*, 1987; Walsh and Harris, 1996a; 1996b; Vaughan *et al.*, 1997a), little or nothing is known about habitat preferences by bats in the Mediterranean region, and specifically Italy.

Because of their peculiar climatic and ecological features (e.g. Blondel and Aronson, 1999), Mediterranean countries differ remarkably from the other European areas where most data on habitat use have been gathered. Over the millennia, the Mediterranean landscape has been shaped into a unique mosaic of habitats by the profound influence of more than 300 human generations (Blondel and Aronson 1999). Therefore, it may be inappropriate to apply conservation guidelines devised for other geographical areas in the management of Mediterranean biodiversity, especially to bats.

Although all chiropteran species have been protected by Italian law since 1939, and legal protection has been recently implemented following the EC 'Habitats' Directive, no framework exists for the protection of foraging habitats. Stebbings (1988) emphasised the urgent need to obtain information on ecological requirements of Italian bats, and Hutson et al. (2001) highlight the importance of developing conservation plans for bats in the Mediterranean region.

Bat activity may be successfully surveyed using ultrasonic detectors (e.g. Kunz and Brock, 1975; Rydell *et al.*, 1994; Walsh *et al.*, 1995; Walsh and Harris, 1996a; 1996b; Vaughan *et al.*, 1997a). To determine species habitat use, objective and quantitative identification methods are recommended, otherwise surveys may lead to serious misinterpretation (Vaughan *et al.*, 1997b; Jones *et al.*, 2000). Italy is an area of high bat species diversity, the occurrence of 31 bat species being in fact documented by historical or recent observations (e.g. Lanza 1959, Mitchell-Jones *et al.* 1999, Russo and Jones 2000). Such a high number of species makes the acoustic identification of bats in flight an especially challenging task. Therefore, it is important to rely on a discrimination method that makes it possible to quantify the degree of correct identification (Zingg 1990, Vaughan *et al.* 1997b, Parsons and Jones 2000, Russo and Jones *in press*).

The aim in this project was to determine the exploitation of foraging habitats by bats in Southern Italy – a typically Mediterranean area – as revealed by acoustic surveys. I aimed primarily to identify those habitats that merit priority conservation measures. This is the first study on bat habitat use ever conducted in the region.

6.3. – Methods

6.3.1. – Study area

The study area lay between latitudes 41°20' and 40°15'N, i.e. it was mostly confined to the Campania region (SW Italy); only two sites were chosen slightly further north, in southern Lazio (Circeo National Park). The area I investigated occupies a central position in the Mediterranean, being located on the boundary of two of the quadrants (NW, NE) in which the Mediterranean Basin may be divided in biogeographical terms (Blondel and Aronson, 1999). The following ten habitat types occurring in Southern Italy were investigated as being representative:

1. Lakes. Five out of 6 replicates chosen were artificial basins. Mean elevation of sites 639 m a.s.l. (range 86-1040 m).
2. Rivers. Sampling focused on main rivers. Streams, although quite common in southern Italy, mostly dry up in summer and were not considered. Mean elevation of river transects 84 m a.s.l. (range 18-160 m).
3. Beech woodlands. These are the typical high-altitude woodlands in the study area. Mean elevation 1259 m a.s.l. (range 1180-1340 m). Mature trees were dominant at all sites.
4. Sweet chestnut *Castanea sativa* woodlands managed for chestnut production. They constitute a traditional form of chestnut woodland management in the study area, and are often characterised by mature trees. Undergrowth is normally either poor or absent as it is removed to facilitate chestnut cropping. Mean elevation 692 m a.s.l. (range 563-840 m).
5. Mediterranean macchia (cf. Blondel and Aronson, 1999): evergreen shrublands characterised by the occurrence of sclerophyllous species such as *Myrtus communis*, *Pistacia lentiscus*, *Arbutus unedo*, *Quercus coccifera*, *Laurus nobilis*, etc. Mean elevation was 369 m a.s.l. (range 5-600 m).

6. Arable land. Farmland in the study area was generally characterised by a relatively complex mosaic of fields separated by tree lines, hedges, canals, etc. Mean elevation 96 m a.s.l. (range 40-200 m).
7. Rural towns. Generally structured in an older centre surrounded by modern settlements. Towns mostly had street lighting and comprised gardens and small fields. Mean elevation 477 m a.s.l. (range 150-700 m).
8. Olive groves. Mean elevation 336 m a.s.l. (range 160-576 m).
9. Mediterranean and sub-Mediterranean woodlands. Low and medium-altitude broadleaved woodlands, dominated by *Quercus ilex* (Mediterranean woodlands *sensu stricto*); mixed deciduous forests of *Alnus cordata, Ostrya carpinifolia, Castanea sativa*; deciduous oak forests dominated by either *Quercus cerris* or *Q. pubescens.* Mean elevation 337 m a.s.l. (range 34-895 m). Mature woodland occurred at all sites.
10. Conifer (*Pinus* spp.) plantations. Four out of six sites were located along the coast at sea level. Median elevation 5 m a.s.l. (range 0-510 m).

6.3.2. – Sampling design and sound recording

Sixty sites – 6 replicates of each habitat type – were chosen. They were large enough to enable a 2-km transect to be walked in a completely homogeneous habitat. Sampling was conducted in 1998 and 1999, from May to October. Each year, 30 sites were visited in a random order to avoid any seasonal influence on sampling. Transects were visited in advance in daylight, their length carefully measured and when necessary trees and other conspicuous objects along the path were marked with reflective tape to make navigation at night easier. When recording, the use of lights was minimised to avoid any interference with bat activity. Following methods of Vaughan *et al.* (1997a), care was taken in walking at a constant speed, and the transects were covered in 45 minutes each starting 30 minutes after sunset. On rare occasions, when transects had to cross habitat interruptions (e.g. clearances in woodland), recording was suspended for not more than 5 minutes. Sites were chosen as far apart as possible, and in most cases an inter-site distance of at least 4 km could be maintained. Before starting and at the end of each transect, I measured air temperature (C°) to the nearest 0.1°C with a digital thermometer and estimated wind speed according to the Beaufort scale. For each transect, a mean value of these variables was calculated and used in data analyses. Percent cloud cover was also estimated at the start of each transect. The percentage of the moon face illuminated on each night was obtained from Whitaker (1998; 1999).

An S25 bat detector (Ultra Sound Advice, London) was kept switched to frequency division, and its HF output was connected to a PUSP (Portable Ultrasound Processor, Ultra Sound Advice, London). Whenever a bat pass – i.e. a series of clicks heard in frequency division as a bat flew within range (Fenton, 1970) – was detected, the PUSP was triggered manually, sampling a 2 s sequence of calls at a rate of 448 kHz and time-expanding it (10x). The corresponding sample of 20 seconds was automatically downloaded and recorded on one channel of Sony Metal XR cassettes by means of a Sony Professional Walkman WM D6C. The S-25 frequency division output was also recorded on the other tape channel. The S25 microphone has a sensitivity of –57dB±3dB (ref. 1V/µbar) from 20-120 kHz. Because it is not possible to time-expand continuously (e.g. Jones *et al.* 2000), while downloading I could not expand any further incoming signal; additional bat passes were counted from frequency division recordings. I made no attempt to identify species or even genera from frequency-divided calls because the high species diversity of the study area would inevitably result in high misclassification.

6.3.3. – Sound analysis and species identification

The recordings were analysed with the software BatSound release 1.0 (Pettersson Elektronik AB, Uppsala). I used a sampling frequency of 44.1 kHz, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis. A 112 Hz frequency resolution was obtained for spectrograms and power spectra. One echolocation call from each bat pass was analysed. A quantitative method (Russo and Jones, *in press*; chapter five) was devised to identify the 21 bat species that are known to occur in the study area (Lanza, 1959; Mitchell-Jones et al., 1999; Russo and Jones, 2000; D. Russo, *unpublished data*). Of the *Myotis* species documented for the area, only *Myotis bechsteinii* – rare throughout Italy (Vergari et al., 1998) – was not included because the latest report dates back to the nineteenth century (Costa, 1839), and subsequent findings are limited to skulls of undetermined age from karstic caves (Russo and Mancini, 1999). Although no direct observation of *Nyctalus leisleri* is available for the area, recent recordings of time-expanded echolocation calls brought strong evidence of its occurrence (D. Russo, *unpublished data*). This species was therefore covered by the identification function. The possible presence of *Nyctalus lasiopterus*, rare in Italy (Vergari et al., 1997), was not taken into account because only one observation – obtained with a heterodyne detector (L. Fornasari, *pers. comm.*) – exists for the area (Zava et al., 1996). After Jones and Barratt (1999), in this study pipistrelles of the 45 kHz phonic type (Jones and Parijs, 1993) are referred to as *Pipistrellus pipistrellus*, and those of the 55 kHz phonic type are termed *Pipistrellus pygmaeus*.

Following Vaughan et al. (1997b), I devised two separate quadratic discriminant functions with cross-validation to identify bats in flight – one for bats emitting FM calls, the other for those producing FM/QCF calls. They were developed from recordings of echolocation calls of 774 Italian bats (mainly from the south) of known identity. The model for bats emitting FM calls relied on start frequency, end frequency, frequency of maximum energy, and duration of the calls (Russo and Jones, *in press*). It covered the following species (corresponding classification rates are in brackets): *Myotis capaccinii* (88%), *Myotis daubentonii* (87%), *Myotis emarginatus* (67%), *Myotis nattereri* (75%), *Myotis myotis* (67%), *Myotis blythii* (53%), *Plecotus auritus* (77%), *Plecotus austriacus* (73%), *Barbastella barbastellus* (90%). Unlike the function described by Russo and Jones (*in press*), the one here applied did not cover *Myotis mystacinus*, a species not observed in Campania so far. Had *M. mystacinus* be recorded, it would have been probably classified as *M. daubentonii*, *M. blythii* or other *Myotis* species (Russo and Jones, *in press*). When *M. mystacinus* recorded in Abruzzo (central Italy) were added to my model, they were frequently misclassified (classification rate was 38%; Russo and Jones, *in press*). The following FM/QCF species were identified with a function (Russo and Jones, *in press*) relying on end frequency, centre frequency, duration, and inter-pulse interval (corresponding classification rates are in brackets): *Pipistrellus kuhlii* (98%), *P. pipistrellus* (98%), *P. pygmaeus* (89%); *Hypsugo savii* (97%), *Eptesicus serotinus* (80%), *Nyctalus noctula* (93%) *N. leisleri* (77%) and *Miniopterus schreibersii* (98%). *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros*, *Rhinolophus euryale* and *Tadarida teniotis* were easily identified from the frequency of maximum energy of their echolocation calls. A number of *Myotis* passes were not identified to species level, and classified as 'unidentified *Myotis*'. This happened:

- when call structure was clearly that of *Myotis* bats but signals were too faint to take accurate measurements. In particular, start frequency – an important variable for DFA identification – was most often affected by poor recording quality.
- when the DFA response attributed calls to *M. blythii*. For this species, the DFA model adopted provided the lowest correct classification (53%, about 1 in 2 calls was misclassified), a result I judged not sufficiently reliable to conduct confident analysis of habitat use to species level. Because the species appears to be relatively common in southern Italy, I preferred to keep it in the discriminant model because its removal

would cause a higher and uncontrollable misclassification rate, as well as an overestimate of the identification performance for the other *Myotis* bats.

When *Pipistrellus* spp. social calls were recorded, they were employed for identification since their structure is diagnostic (Barlow and Jones, 1997; Russo and Jones, 1999; Russo and Jones, 2000).

6.3.4. – Data analysis

Bat activity was measured as the number of bat passes recorded. Time-expanded bat passes were pooled with those recorded only in frequency division for total activity analysis. As in other studies (e.g. Furlonger et al., 1987; Walsh et al., 1995; Vaughan et al., 1997a), feeding buzzes (call sequences produced by bats that attempt prey capture; Griffin et al., 1960) were commonly recorded and their total number analysed as a measure of foraging attempt.

Analyses of variance and covariance (ANOVA, ANCOVA) were applied to analyse both overall activity and that of species frequently recorded. The overall number of feeding buzzes – a measure of foraging attempt – was also tested for effects of habitat and covariates. Preliminary data exploration (scatter plots, correlation analysis) showed that cloud cover and moon phase had no effect over bat activity: hence, these variables were not used in further analyses. In most cases the samples conformed to ANOVA and ANCOVA assumptions (Huitema, 1980) after appropriate transformation (square-root, $\log_e$, square-root $\log_e$). In these cases data were tested for differences between habitat types, and variables which appeared to influence activity were entered as covariates in an ANCOVA. In using ANCOVA the covariate has to be measured on a continuous scale (Huitema, 1980). Beaufort scale is not continuous (each value corresponding to a defined wind speed range), so in order to use wind speed as a covariate, the Beaufort values estimated in the field were converted to the corresponding range means in km/h. A mean value respectively of wind speed and temperature obtained at the start and at the end of each transect was calculated and used in the analyses. Because elevation was actually a feature for some of the habitat types considered and not independent from the analysis 'treatment', it could not be used as a covariate (Huitema, 1980). Interactions between 'habitat type' and covariates were tested for, and removed from models because in no case did they reach significance (Aitkin et al., 1989). Likewise, covariates were removed from models where their effect was not significant, and when no covariate was left in the model, a one-way ANOVA was applied. Only the analysis final results are presented here.

When a significant habitat effect was detected, multiple post-hoc comparisons on means adjusted by ANCOVA were performed with the Bryant-Paulson Tukey test (Day and Queen, 1989); ANOVA was followed by a Newman-Keuls test. The Ryan-Joiner test was applied to verify data conformity to normal distribution, and Fmax and Levene tests were used to test for homogeneity of variance.

The species richness of the foraging bat community was analysed in two ways: the effect of habitat on the mean number of species detected in each habitat (mean S) was tested with an ANOVA, and the total number of species recorded in each habitat type (total S) was compared between habitats.

Means are presented ± SD. All analyses, except Fmax, Levene and Newman-Keuls and Bryant-Paulson Tukey tests, were performed with MINITAB release 9.2.

6.4. – Results

6.4.1. – Bat activity

Overall, 4104 bat passes were recorded, and 3466 of them (84.5%) were identified to species (Tab. 6.1). No identification attempt was made for 483 passes recorded only in frequency division and 27 very faint time-expanded sequences. Finally, 128 passes (32.8% of which classified as *M. blythii*) were attributed to the 'unidentified *Myotis*' category. I also recorded 569 feeding buzzes (i.e. on average 13.9% of bat passes included a feeding buzz). A strong correlation (rs = 0.85, n = 60, p<0.001) between numbers of feeding buzzes and bat passes (i.e. between foraging attempts and activity rates) recorded at each site confirmed that the number of bat passes was a reliable estimator of foraging activity. The species most frequently recorded were (in decreasing order): *P. kuhlii*, *H. savii*, *P. pipistrellus*, *M. daubentonii*, *P. pygmaeus*, *T. teniotis*, *M. capaccinii* and *M. schreibersii* (Tab. 6.1). The numbers of passes recorded from all other species (Tabs. 6.1, 6.2) were too low to be used for quantitative analysis.

Overall bat activity (Fig. 6.1) differed significantly between habitat types ($F_{9,50}$=3.91, p<0.001, log transformed data). High levels of activity were observed on rivers and lake shores. Likewise, the number of feeding buzzes recorded in such habitats was the highest ($F_{9,50}$=8.61, p<0.001, log transformed data; Fig. 6.2). Relatively large numbers of bat passes and of foraging attempts were recorded at some rural town and arable land sites, but this trend was not confirmed statistically on account of the large sample variability (Fig. 1).

P. kuhlii occurred in all habitats (Fig. 6.3), often with high activity levels. Although some significant differences were detected between habitats ($F_{9,50}$=3.91, p<0.005, square root transformed data; Fig. 6.3), the species was quite generalist in exploiting foraging habitats. Feeding buzzes of *P. kuhlii* (n = 186) were recorded at 83.3% of sites.

H. savii activity increased significantly with temperature (Tab. 6.3). Once activity was corrected for temperature, it was significantly higher on lake and river shores (Fig. 6.4). Conifer plantations were the least used habitat. Only 34 *H. savii* feeding buzzes were recorded, mainly (76.5%) on lake shores.

Like *P. kuhlii*, *P. pipistrellus* also showed limited, although significant, differences in activity ($F_{9,50}$= 4.40, p<0.001, log transformed data; Fig. 6.5), which was relatively higher for lakes and beech woodlands. Feeding buzzes (n = 80) were recorded at 45 sites in all habitats. Unlike *P. kuhlii*, *P. pipistrellus* made quite a large use of beech woodlands: in al, in this habitat I recorded 95 passes from the latter species vs. 42 from the former (Figs. 6.3, 6.5).

P. pygmaeus proved more selective ($F_{9,50}$=4.73, p<0.001; square roots of log transformed data). Activity was significantly higher over rivers and in chestnut woodlands (Fig. 6.6). No passes were recorded in rural towns and olive groves. Only 17 *P. pygmaeus* feeding buzzes were recorded. *M. schreibersii* also was most active in rivers ($F_{9,50}$=2.78, p<0.05, square roots of log transformed data); it was never recorded in Mediterranean macchia and arable land (Fig. 6.7).

T. teniotis activity varied considerably within habitats. I recorded a low activity in all habitats except lakes (mean n of passes 9.7±18.7, range 0-47) and Mediterranean and sub-Mediterranean woodlands (4.5±6.6, range 0-16). No passes were recorded in beech woodlands, arable land and rural towns. Activity did not differ significantly between habitats ($F_{9,50}$=1.57, NS; square roots of log transformed data). In all cases *T. teniotis* clearly flew high up, i.e. away from clutter, and in woodlands foraged over the canopy.

The activity of *Myotis* spp. (*Myotis* passes identified to species + 'undetermined *Myotis*') differed significantly between habitats ($F_{9,50}$=14.7, p<0.001; square root transformed data; Fig. 6.8). It was highest along lake and river shores (92% of *Myotis* passes were recorded at water sites), and also slightly higher in chestnut and beech woodlands. No *Myotis* passes were heard in Mediterranean macchia and rural towns.

Despite data transformation, the number of passes of *M. daubentonii* and *M. capaccinii* failed to meet the analysis of variance assumptions. However, it was obvious that rivers and lakes were most used by both species, only a few passes having been recorded in other habitats (woodlands; Fig. 6.9). Samples obtained from rivers and lakes met the analysis of variance requirements, and so comparison of activity was limited to these habitats for both species. Preliminary data exploration suggested some influence of wind speed, which was then entered as a covariate. Once the significant effect of wind speed had been adjusted for by ANCOVA, *M. capaccinii* activity showed no difference between lakes and rivers, unlike *M. daubentonii* which preferred rivers (Tab. 6.4).

Because *M. capaccinii* activity did not differ between rivers and lakes, data from all water sites could be lumped together for correlation analysis and the occurrence of a significant, high negative correlation (r_s = - 0.95, n = 12, $p<0.001$) between activity and wind speed was confirmed (Fig. 6.10). Feeding buzzes of both species were detected only over lakes (*M. daubentonii* n = 8, *M. capaccinii* n = 24) and rivers (*M. daubentonii* n = 14, *M. capaccinii* n = 7).

6.4.2. – Species richness

The mean number of foraging species differed significantly between habitats ($F_{9,50}=5.57$, $p<0.001$; square root transformed data; Fig. 6.11). On average, rivers had significantly more species than all habitats except lakes (Tab. 6.5). Lakes and chestnut woodland also had a high mean number of species, although the trend was not significant. Mediterranean macchia, arable land and conifer plantations had the lowest mean number of species.

Large total numbers of species (>10) were recorded in chestnut woodlands, followed by rivers, beech woodlands, lakes, Mediterranean and sub-Mediterranean woodlands and olive groves (Table 6.5). A considerable percentage (>40%) of the species found in these habitats are classified as endangered or vulnerable in Italy. The remaining habitats had a lower total number of species, mostly belonging to the 'low risk' category.

6.5. – Discussion

6.5.1. – Effect of habitat on bat activity

The identification rate I achieved is similar to that (83 %) obtained by Vaughan et al. (1997a), who used an identical sampling method for their study on British bats. The importance of riparian foraging habitats found in my study confirms findings for other geographical areas (e.g. Rydell et al., 1994; Walsh et al., 1995; Grindall et al., 1999; Vaughan et al., 1997a, Racey, 1998). Such habitats support a higher prey insect density than other habitat types (e.g. Barclay, 1991). In this study, high levels of activity of *Myotis* bats, *H. savii*, *P. pygmaeus* and *M. schreibersii* were recorded over rivers and lakes, and generalist species such as *P. kuhlii* and *P. pipistrellus* were also abundant at water sites. Vaughan et al. (1997a) showed rivers and lakes to be the main foraging habitats for British *P. pygmaeus*. At a British riparian site, however, Warren et al. (2000) found that *P. pygmaeus* was scarce, and *P. pipistrellus* frequent. As these authors pointed out, elevation (as well as other unknown environmental factors) might influence the relative distribution of the two pipistrelle species. The use of rivers by *M. schreibersii* may not be limited to foraging, as this species seems to follows rivers as navigation landmarks (Sierra-Cobo et al., 2000).

The highly opportunistic choice of foraging sites by *P. kuhlii* and *P. pipistrellus*, also observed in England in the latter species (Vaughan et al., 1997a), is probably made possible by their plasticity in echolocation (Schnitzler et al.1987, Kalko and Schnitzler 1993, Jones and Parijs 1993). This feature may allow the bats to exploit a variety of differently structured foraging habitats (Norberg and Rayner, 1987) and feed upon a larger prey spectrum (Swift et al., 1985; Beck, 1995; Barlow, 1997; Arlettaz et al., 2000). Both species frequently feed by street lamps (e.g. Haffner and Stutz; 1985/6; Blake et al., 1994; Rydell, 1992; Russo and Jones, 1999). In Italy, *P. kuhlii* is the most abundant bat species (Lanza, 1959). *P. pipistrellus* is widespread and abundant across its European range (Mitchell-Jones et al., 1999). The 'success' of *P. pipistrellus* and *P. kuhlii* may be due to the observed lack of habitat preferences, plasticity in roost selection (Schober and Grimmberger, 1997) and 'r-selected features' (Begon et al., 1986) – i.e. short life span, first parturition at one year of age and frequent birth of twins (Arlettaz et al., 2000). The observed difference in the use of beech woodlands (all sites occurring over 1000 m a.s.l) between *P. pipistrellus* and *P. kuhlii* may be determined by an elevational effect. In fact, *P. kuhlii* is associated with lower altitudes (Schober and Grimmberger, 1997; Vernier and Bogdanowicz, 1999).

Although during this study I failed to record *T. teniotis* in beech woodlands, arable land and towns, in Campania and Abruzzo (central Italy) it has been observed foraging in high flight over villages, beech woodlands, cultivated fields – especially if bordered by illuminated roads – and even large cities (Russo and Mastrobuoni, 1998). Ahlén (1990) reported that *T. teniotis* forages over illuminated villages. This species may tend to forage in built-up sites later at night, as occasionally observed in some of the towns I visited (D. Russo, *pers. obs.*), when the temperature is appreciably higher than elsewhere.

6.5.2. – Other effects on bat activity

In this study, no influence of cloud cover and moon phase over bat activity was detected, as found by other authors (Geggie and Fenton, 1985; Negraeff and Brigham, 1995; Vaughan et al., 1997a; Gaisler *et al.*, 1998). Unlike bats in England (Vaughan et al., 1997a), those from southern Italy were not influenced by temperature, with the sole exception of *H. savii.* The mild ambient temperatures I recorded (>10°C at all study sites but one, mean 18.3°C) is likely to have had limited or null influence on insect distribution (Taylor, 1963; Williams, 1940; 1961) and, consequently, on bat activity (Catto et al., 1995; Walsh et al.,1995; Vaughan et al., 1997a). In a bat activity survey in a Czech urban area (Gaisler et al., 1998) in which no field work was conducted at temperatures < 10°C, a thermal influence emerged only for the activity of *E. serotinus*. Since my surveys started half an hour after sunset and lasted less than one hour, it cannot be ruled out that temperature might affect activity later in the night. In Ireland, *N. leisleri* activity was correlated with temperature only after the first third of the night (Shiel and Fairley, 1998). The sensitivity to wind of *M. capaccinii* and *M. daubentonii* may be due to the fact that on windy days bats prefer sheltered foraging sites (Boonman, 1996), where insect density is higher (Lewis and Stephenson, 1966; Lewis, 1967; 1969). Prey occurrence at windy sites may be too low to meet the high prey capture rate pursued by the bats (see e.g. Kalko and Braun, 1991 for *M. daubentonii*). Wind also increases the number and size of ripples on water surface, and thus it may affect echolocation in *M. capaccinii* and *M. daubentonii*, which both generally hunt very close to the water surface (Jones and Rayner, 1988; Kalko and Schnitzler, 1989; Barataud, 1996). *M. daubentonii* tends to avoid turbulent or cluttered water surfaces which produce ultrasonic noise and confusing echoes (Boonman et al., 1998; Rydell et al., 1999; Warren et al., 2000).

6.5.2. – Species richness

Data on infrequently recorded species were insufficient for confident analysis of habitat preference but were used to assess the number of species found in each habitat. Rare species may not be detected in transect surveys (see Kunz *et al.* 1996). Although the species I recorded infrequently were often those occurring in lower numbers in the study area – such as *B. barbastellus* and *M. nattereri* – this was not always the case. In the study area, *R. ferrumequinum* and *R. hipposideros* are widespread, although generally with rather low densities (D. Russo *unpublished data*). *Plecotus* spp. and rhinolophids are difficult to detect because their echolocation calls are difficult to detect (Waters and Jones

1995, Vaughan et al., 1997a; Gaisler et al., 1998) and may have been overlooked. However, in my study I could compare the species richness of the foraging bat community across habitats as the above limitations probably affected all transects equally. The importance of riparian habitats for bats was confirmed by the high numbers of species observed on river and lake shores. Many species featured in all broadleaved woodland types although in these habitats activity was considerable only for a few of them – especially *P. pipistrellus* (Fig. 6.5), *P. pygmaeus* (Fig. 6.6) and *Myotis* spp. (Figs. 6.8, 6.9). Radio-tracking revealed that *R. euryale* in southern Italy foraged primarily in broadleaved woodland and significantly in olive groves (Russo *et al.* a, in press), and avoided urban sites, open areas and conifer plantations. The present study shows that conifer plantations have limited value for foraging: they were used by few species and even the widespread *H. savii* showed a low activity in this habitat and was recorded only in three out of six conifer sites. Bats may prefer broadleaved woodlands to conifer ones because the former support more prey insects (Waring, 1988; 1989; Entwistle et al., 1996). Mediterranean macchia was the least important natural habitat in terms of bat activity and number of species: tall vegetation and water, both valuable to bats, are scarce in this habitat. Arable land and rural towns were used for foraging only by few 'opportunistic' species, best adapted to anthropogenic habitats. The importance of olive groves was probably enhanced by the traditional management and structural diversity occurring at most sites.

6.5.3. – Managing bat foraging habitats in Mediterranean areas

The protection and correct management of water habitats are undoubtedly key points in planning bat conservation in Mediterranean areas. Italian riverine habitats and fauna are threatened by many factors (e.g. Martino, 1992; Prigioni, 1997) such as pollution, channelisation, dredging, damming, alteration and destruction of riparian vegetation. Degradation of riparian habitats influences their insect communities (Jeffries and Mills, 1990; Fry and Lonsdale, 1991), and consequently foraging bats (Stebbings, 1988; Vaughan et al., 1996). The effect of water eutrophication on foraging bats is still unclear (Racey, 1998). Vaughan *et al.* (1996) found that the overall bat activity, as well as that of both pipistrelle cryptic species, was affected negatively by sewage effluents, which favoured *M. daubentonii*. Racey *et al.* (1998) showed that water nutrient enrichment favoured both pipistrelles and *M. daubentonii*. European populations of *M. daubentonii* may be growing as a result of eutrophication (Kokurewicz, 1995). No information on the effect of water enrichment on more vulnerable bat species is available.

Riparian vegetation should be protected and encouraged because it shelters foraging sites from the wind, improving their quality (Zahn and Maier, 1997). Bank vegetation favours the presence of prey insects that are directly associated with the availability of food plants and sheltered sites and mitigates the effect of wind on water turbulence (Peng et al., 1992; Warren et al., 2000). This study showed that *M. capaccinii* – greatly endangered in Europe (Guillén, 1999) – and *M. daubentonii* are wind-sensitive and would probably benefit greatly from conservation of riparian vegetation.

The high temperatures and scarcity of water typical of a Mediterranean summer (Blondel and Aronson, 1999) are likely to enhance the importance of water habitats as providing opportunities for drinking. Bats face the risk of dehydration, especially in summer (Racey, 1998), and this is all the more crucial in the Mediterranean climate. In the beech woodlands and pastures of central Italy, even cattle troughs are important summer drinking sites for many bat species: as many as 11 species have been found to drink regularly at a single pool (D. Russo, *unpublished data*). Favouring even small drinking sites (such as ponds, troughs) may increase the value of arid areas for bat foraging.

Broadleaved woodlands are also important targets for bat conservation in Mediterranean areas as elsewhere. These habitats, together with water sites, were used by a considerable number of threatened bat species (Tab. 6.5). The occurrence of old or dead trees provides tree-dwelling bat species with roosts (Mayle, 1990); and dead wood and undergrowth may sustain prey insects, so that areas of largely or completely unmanaged woodland should be maintained where possible. Because habitat connectivity is important to bats (Walsh and Harris, 1996a), habitat interruptions should be avoided in logging protocols. The size of logged patches should be minimised, and corridors between main blocks of woodland should be maintained. Where feasible, reforestation with broadleaved trees rather than conifers should be preferred (see also Russo *et al.* a, in press). *C. sativa* woodlands managed for chestnut production – where old trees are often present – sustained a significant number of bat species, including several threatened ones. This traditional form of chestnut woodland management, which in some areas of Italy has economical significance, should be encouraged where possible.

I found that some towns and farmland sites had an intense bat activity. The rural towns I investigated were mostly illuminated, and generally scattered with small gardens, orchards and fields. The presence of vegetation may sustain insect prey populations, and lights attract insects and improve the prey capture success rates by bats (e.g. Furlonger et al., 1987; de Jong and Ahlén, 1991). In southern Italy, urban parks and gardens are tending to disappear as towns develop and spread. The negative effects of urbanisation on bats (Kurta and Teramino, 1992, Gaisler et al.1998) might be mitigated by maintaining trees, gardens and small patches of cultivated land.

Throughout Europe, the degradation of farmland associated with intensive agricultural practices is threatening bats (Stebbings 1988), and although no detailed data are available, this is likely to be the case in Mediterranean regions too. Simplification of the agricultural landscape may have adverse effects on both prey availability and the occurrence of linear landscape elements used by bats as commuting landmarks (e.g. Limpens and Kapteyn, 1991). Farmland practices should maximise landscape complexity, favour structural variation and connectivity, and limit the spread of pesticides, which has harmful effects on bat populations (Stebbings, 1988; Hutson et al., 2001). Such considerations also apply to olive groves, which are used by a speciose chiropteran community. Non-intensive, or even organic farming is probably the most promising management option for bat conservation in many areas of the Mediterranean countryside.

Tab. 6.1. Numbers of bat passes recorded in 60 transects.

Species	Number of bat passes counted	% of total
Rhinolophus ferrumequinum	12	0.29
Rhinolophus hipposideros	13	0.32
Rhinolophus euryale	1	0.02
Myotis daubentonii	146	3.56
Myotis capaccinii	92	2.24
Myotis emarginatus	12	0.29
Myotis nattereri	4	0.10
Myotis myotis	6	0.15
Myotis sp.	128	3.12
Nyctalus noctula	8	0.19
Nyctalus leisleri	40	0.97
Eptesicus serotinus	9	0.22
Pipistrellus pipistrellus	412	10.04
Pipistrellus pygmaeus	132	3.22
Pipistrellus kuhlii	1570	38.26
Hypsugo savii	457	11.13
Plecotus auritus	1	0.02
Plecotus austriacus	0	0.00
Barbastella barbastellus	5	0.12
Miniopterus schreibersii	71	1.73
Tadarida teniotis	115	2.80
Unidentified (time expansion)	27	0.66
Frequency division	483	11.77
Total (time expansion)	3621	88.23
Total	4104	100.00

Tab. 6.2. Number of passes of bat species infrequently recorded in each habitat. Abbreviations as in Figs. 1-8.

Species	La	Ri	Bw	Cw	Mm	Al	Rt	Og	Mw	Cp
Rhinolophus ferrumequinum	0	5	0	1	1	0	0	3	2	0
Rhinolophus hipposideros	0	2	2	1	3	0	0	0	5	0
Rhinolophus euryale	0	0	0	0	0	0	0	0	1	0
Myotis emarginatus	3	2	2	2	0	0	0	1	0	2
Myotis myotis	0	3	0	0	0	1	0	2	0	0
Myotis nattereri	0	0	2	2	0	0	0	0	0	0
Nyctalus leisleri	3	3	1	28	1	0	2	1	1	0
Nyctalus noctula	6	0	0	2	0	0	0	0	0	0
Eptesicus serotinus	2	0	1	0	1	2	2	1	0	0
Plecotus auritus	0	0	1	0	0	0	0	0	0	0
Barbastella barbastellus	0	0	1	2	0	0	0	1	1	0
Miniopterus schreibersii	1	42	2	11	0	0	1	9	4	1

Tab. 6.3. ANCOVA for effect of habitat and temperature on activity of *Hypsugo savii*.

Source of variance	D.F.	Adj. MS	*F*	*p*
Habitat	9	4.648	6.29	<0.001
Temperature (covariate)	1	12.751	17.25	<0.001
Error	49	0.739		
Total	59			

Tab. 6.4. ANCOVA for effect of habitat (lakes and rivers) and wind on activity of *M. daubentonii* and *M. capaccinii*. Analysis was conducted on square root-transformed data.

	Myotis daubentonii				*Myotis capaccinii*			
Source of variance	D.F.	Adj. MS	*F*	*p*	D.F.	Adj. MS	*F*	*p*
Habitat	1	8.193	17.04	<0.005	1	0.300	0.29	NS
Wind (covariate)	1	7.103	14.77	<0.005	1	17.135	16.36	<0.005
Error	9	0.481			9	1.047		
Total	11				11			

Tab. 6.5. Bat species recorded in each habitat type, their status in Italy (after Bulgarini *et al.*, 1998), total number of species/habitat (total S) and mean (mean S), SD and range of the number of species detected at each site. Habitat abbreviations as in figure 1. LR = Low Risk; VU = Vulnerable; EN = Endangered.

Species	Status in Italy	Habitat									
		La	Ri	Bw	Cw	Mm	Al	Rt	Og	Mw	Cp
R.. ferrumequinum	VU		✓		✓	✓			✓	✓	
R hipposideros	EN		✓	✓	✓	✓				✓	
R.. euryale	VU									✓	
M. capaccinii	EN	✓	✓		✓						
M. daubentonii	VU	✓	✓	✓							✓
M. emarginatus	VU	✓	✓	✓	✓				✓		✓
M. myotis	VU		✓				✓		✓		
M. nattereri	EN			✓	✓						
N. leisleri	VU	✓	✓	✓	✓	✓		✓	✓	✓	
N. noctula	VU	✓			✓						
E. serotinus	LR	✓		✓		✓	✓	✓	✓		
P. kuhlii	LR	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. pipistrellus	LR	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. pygmaeus	-	✓	✓	✓	✓	✓	✓			✓	✓
H. savii	LR	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. auritus	LR			✓							
P. austriacus	LR										
B. barbastellus	EN			✓	✓				✓	✓	
M. schreibersii	LR	✓	✓	✓	✓			✓	✓	✓	✓
T. teniotis	LR	✓	✓		✓	✓			✓	✓	✓
Total S		12	13	13	14	9	6	6	11	11	8
N EN+VU species		5	7	6	8	3	1	1	5	5	2
(% of total S)		(41.7)	(53.8)	(46.2)	(57.1)	(33.3)	(16.7)	(16.7)	(45.4)	(45.4)	(25.0)
Mean S		6.5	8.2	4.3	5.7	3.0	3.3	3.7	3.8	4.1	3.0
(SD)		(1.5)	(2.5)	(1.0)	(2.7)	(1.4)	(1.2)	(1.0)	(1.2)	(1.5)	(1.8)
Range		5-9	5-12	3-6	3-9	1-5	2-5	3-5	2-5	2-6	1-5

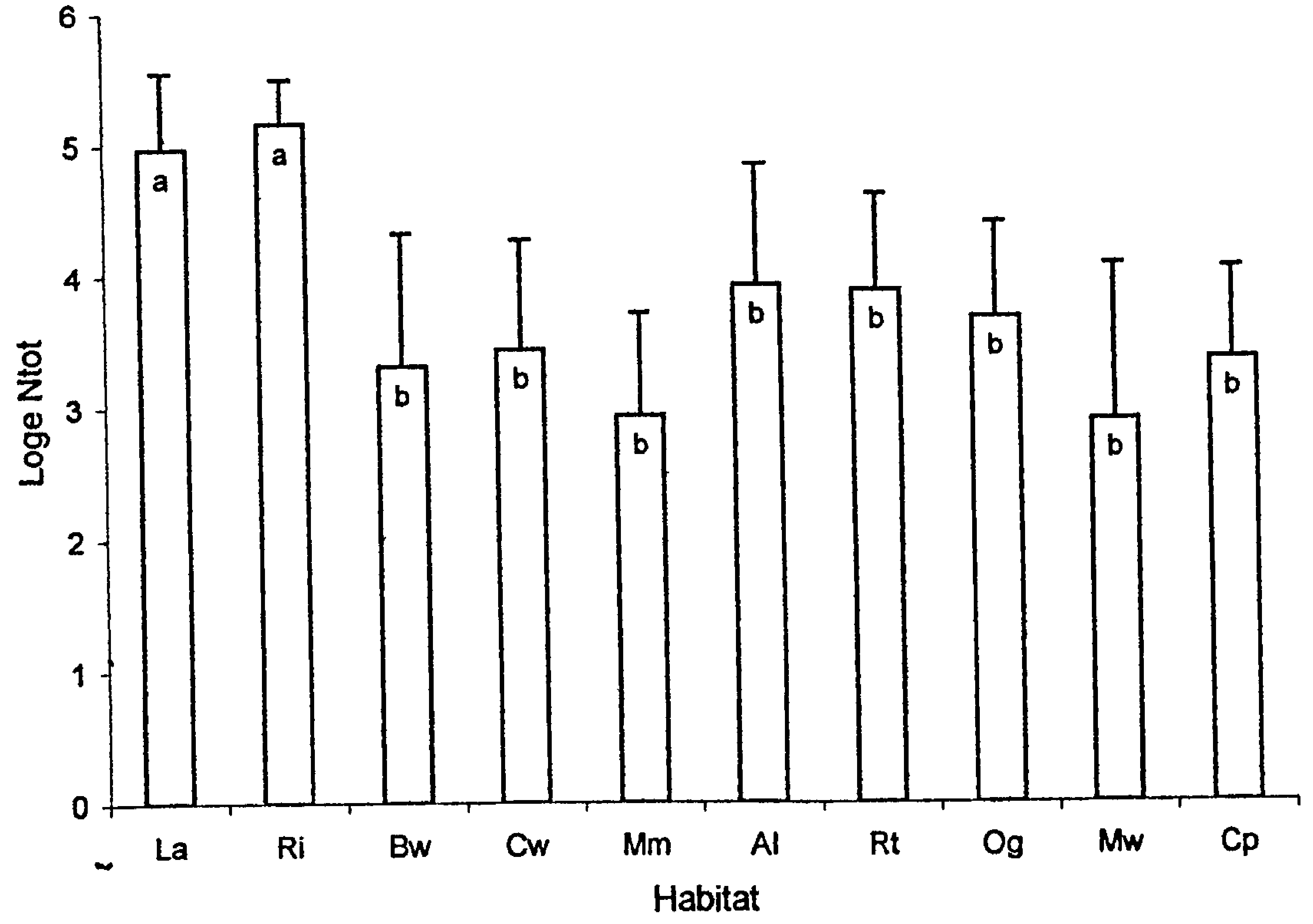

Fig. 6.1. Mean log transformed counts of total bat passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

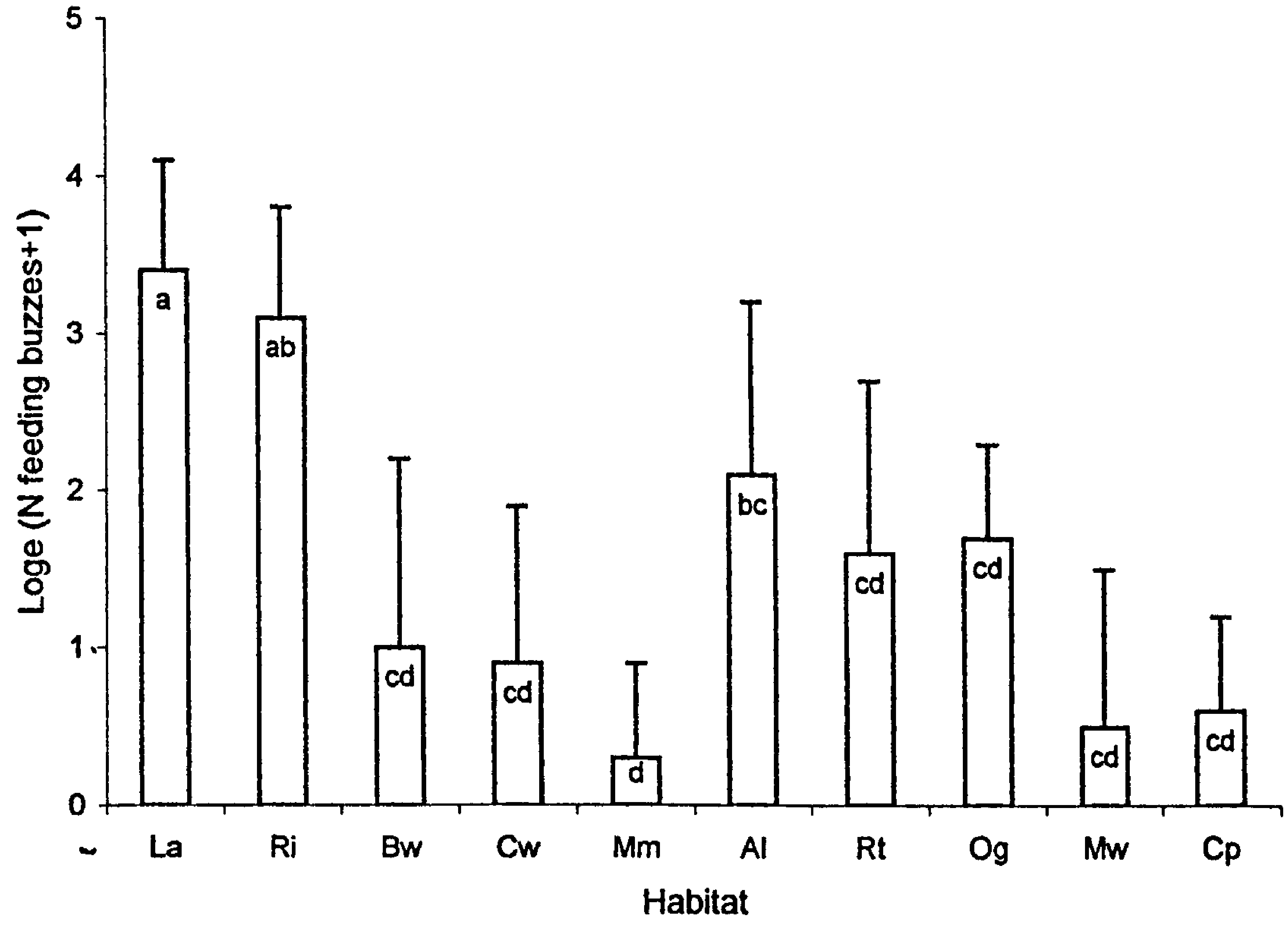

Fig. 6.2. Mean log transformed counts of total feeding buzzes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

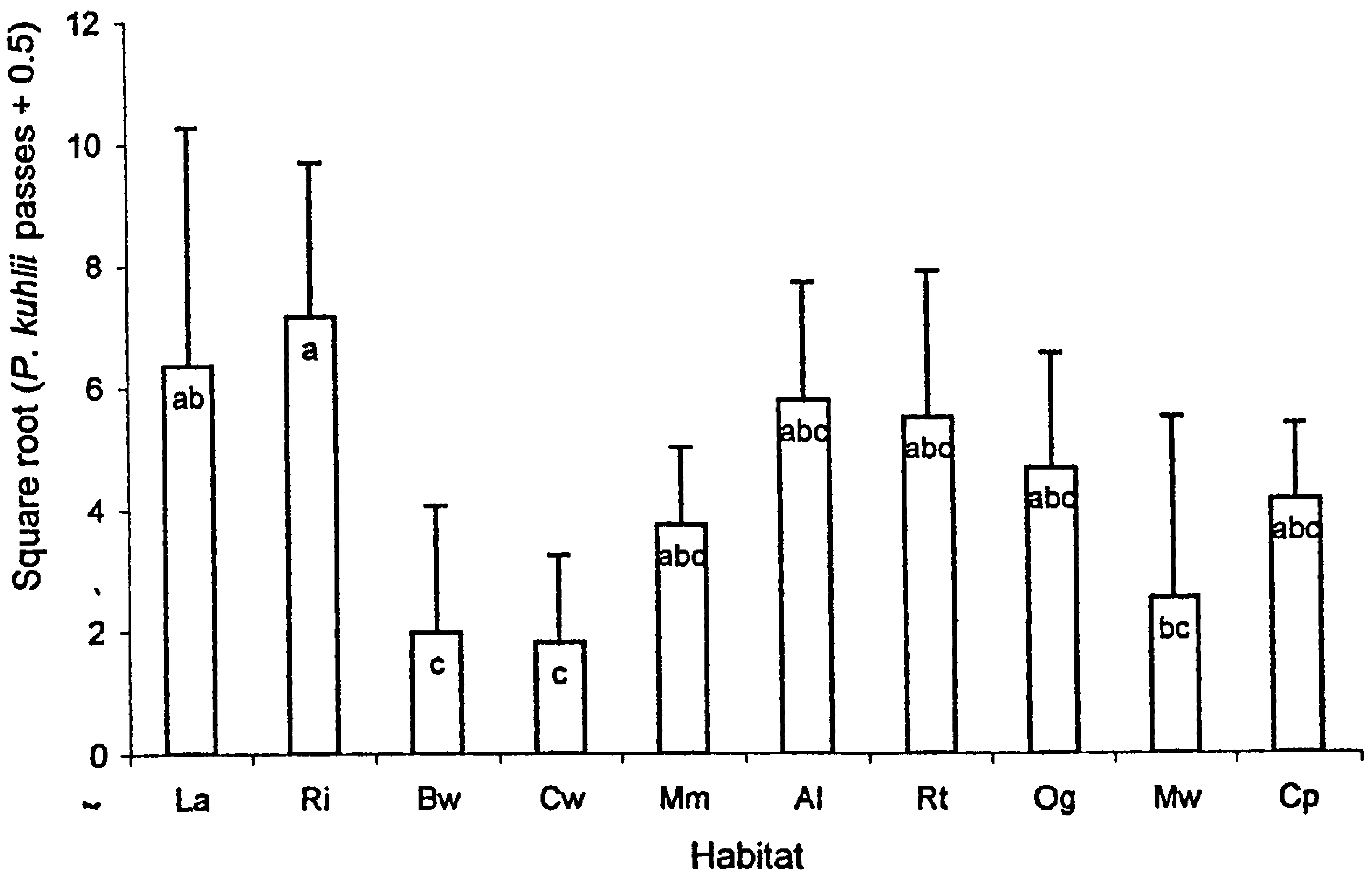

Fig. 6.3. Mean square root transformed counts of *Pipistrellus kuhlii* passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

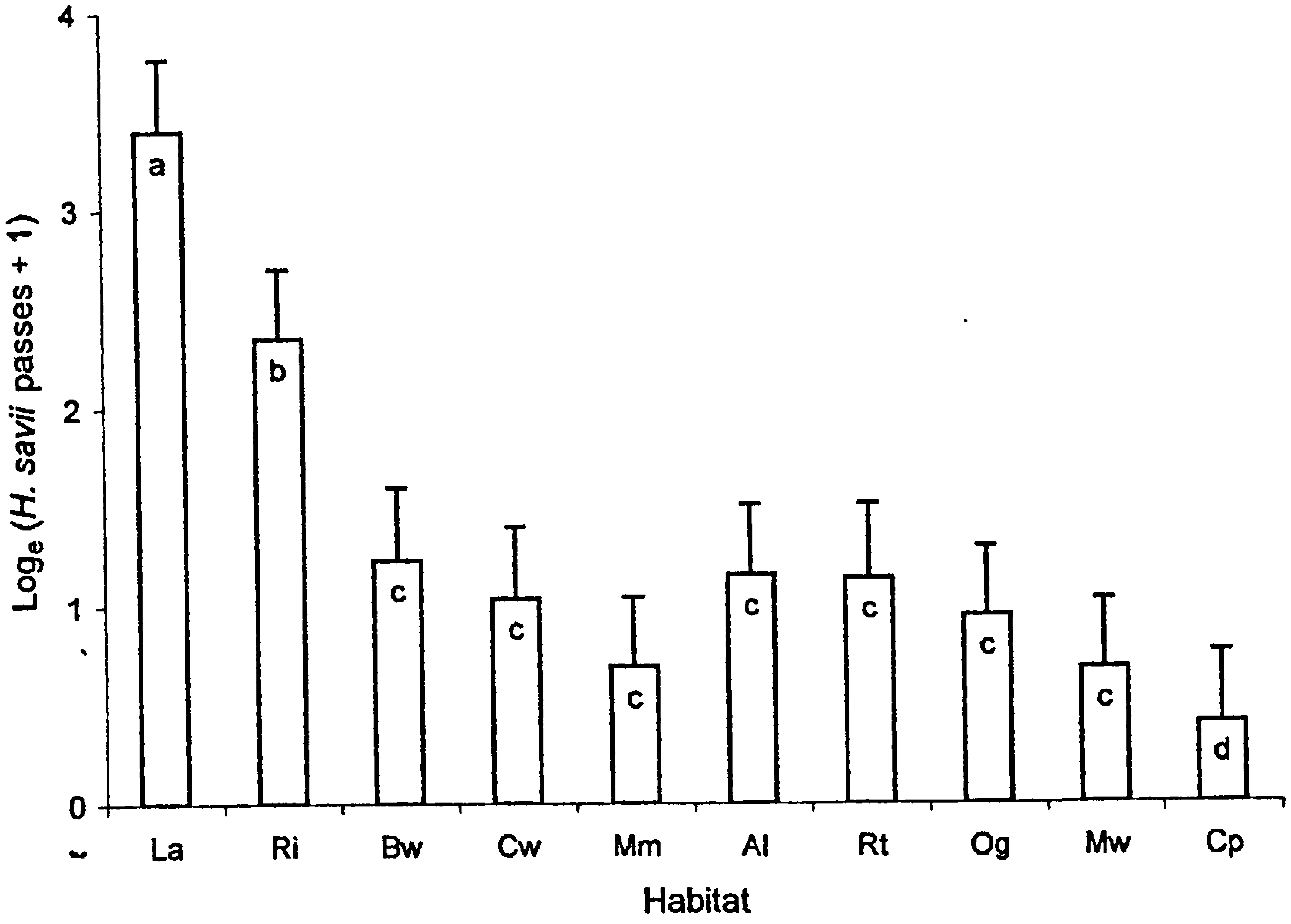

Fig. 6.4. Mean log transformed counts of *Hypsugo savii* passes adjusted for effect of temperature recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

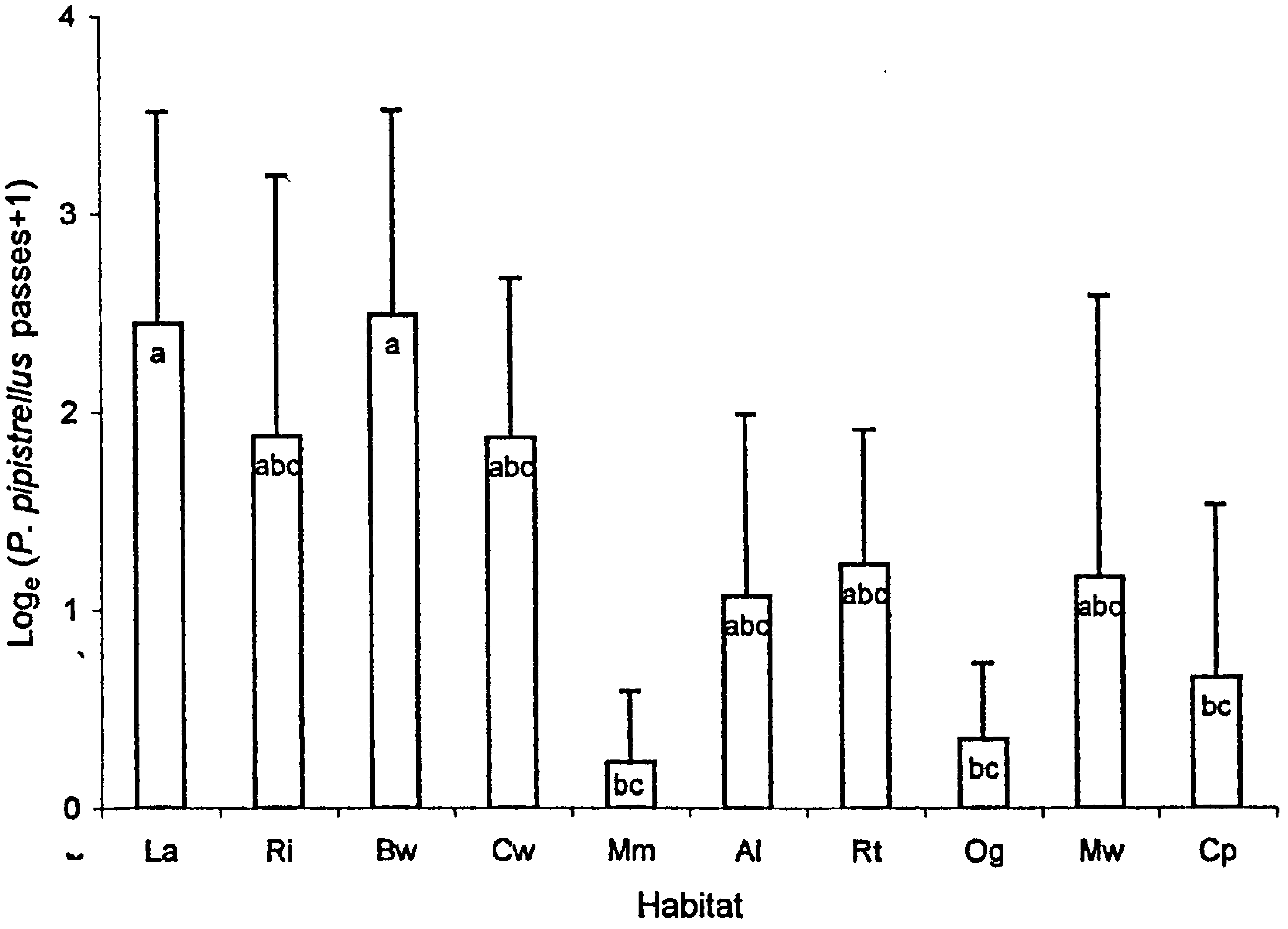

Fig. 6.5. Mean log transformed counts of *Pipistrellus pipistrellus* passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

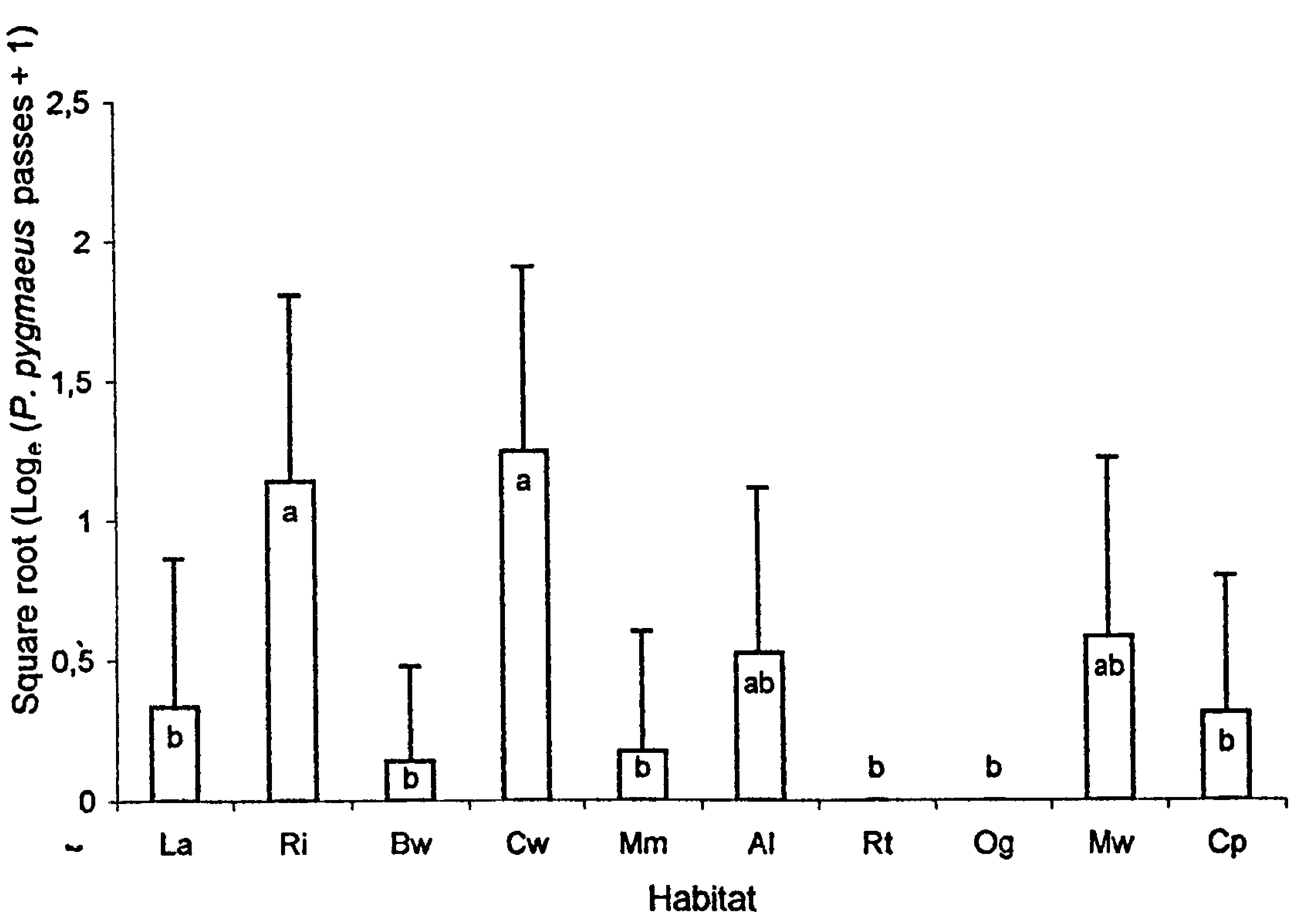

Fig. 6.6. Mean square root log counts of *Pipistrellus pygmaeus* passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

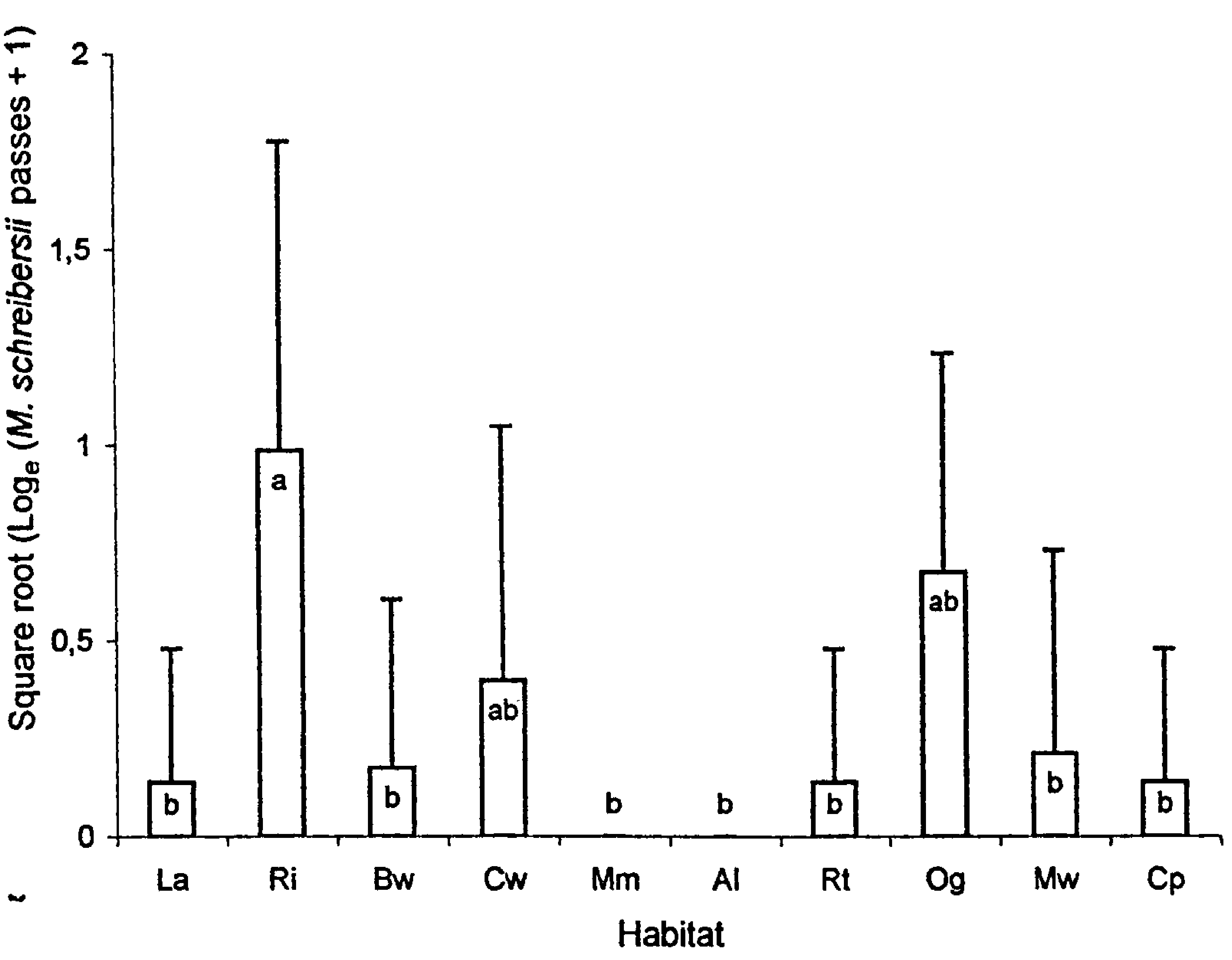

Fig. 6.7. Mean square root log counts of *Miniopterus schreibersii* passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

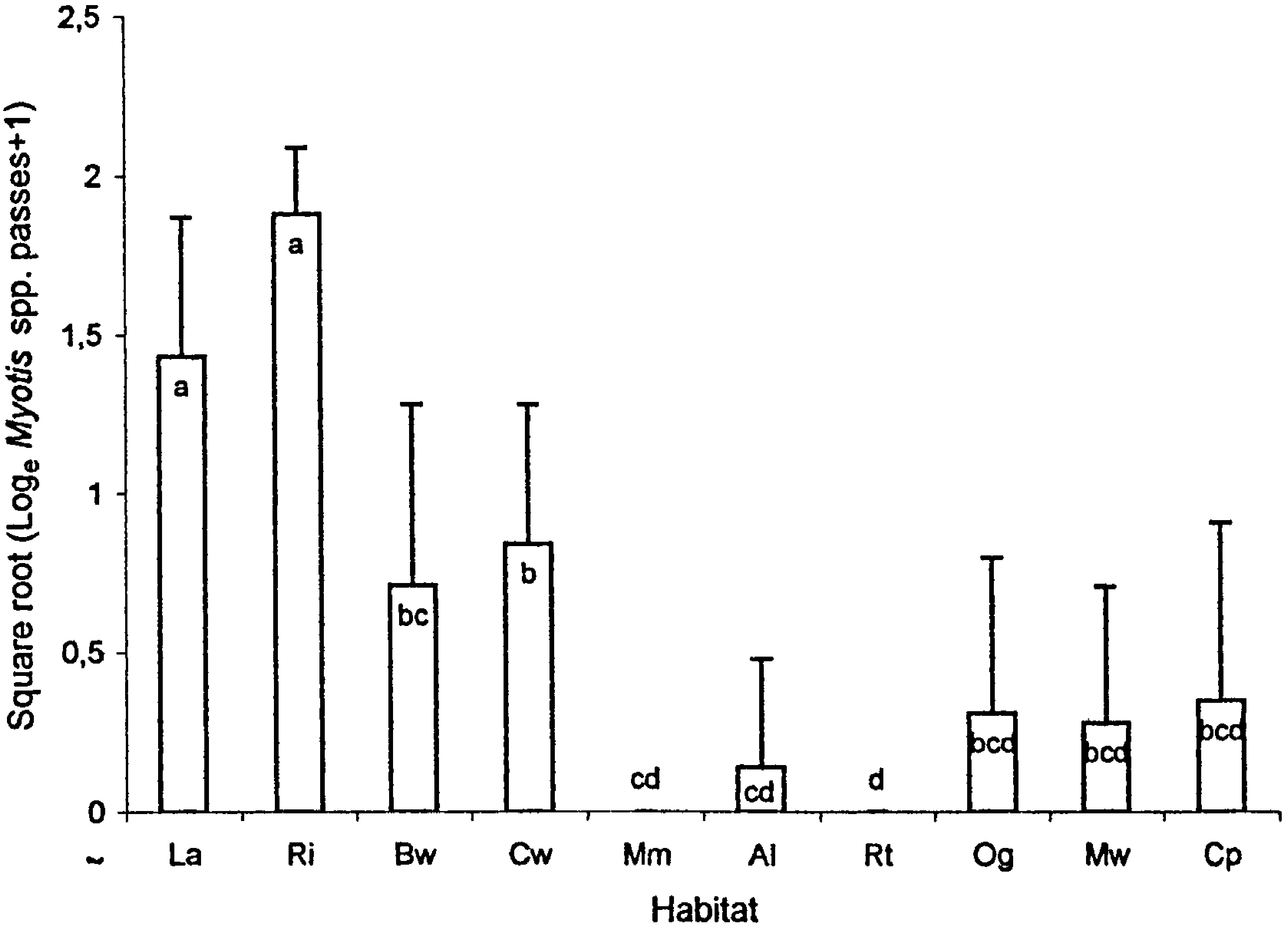

Fig. 6.8. Mean square root log transformed counts of *Myotis* spp. passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

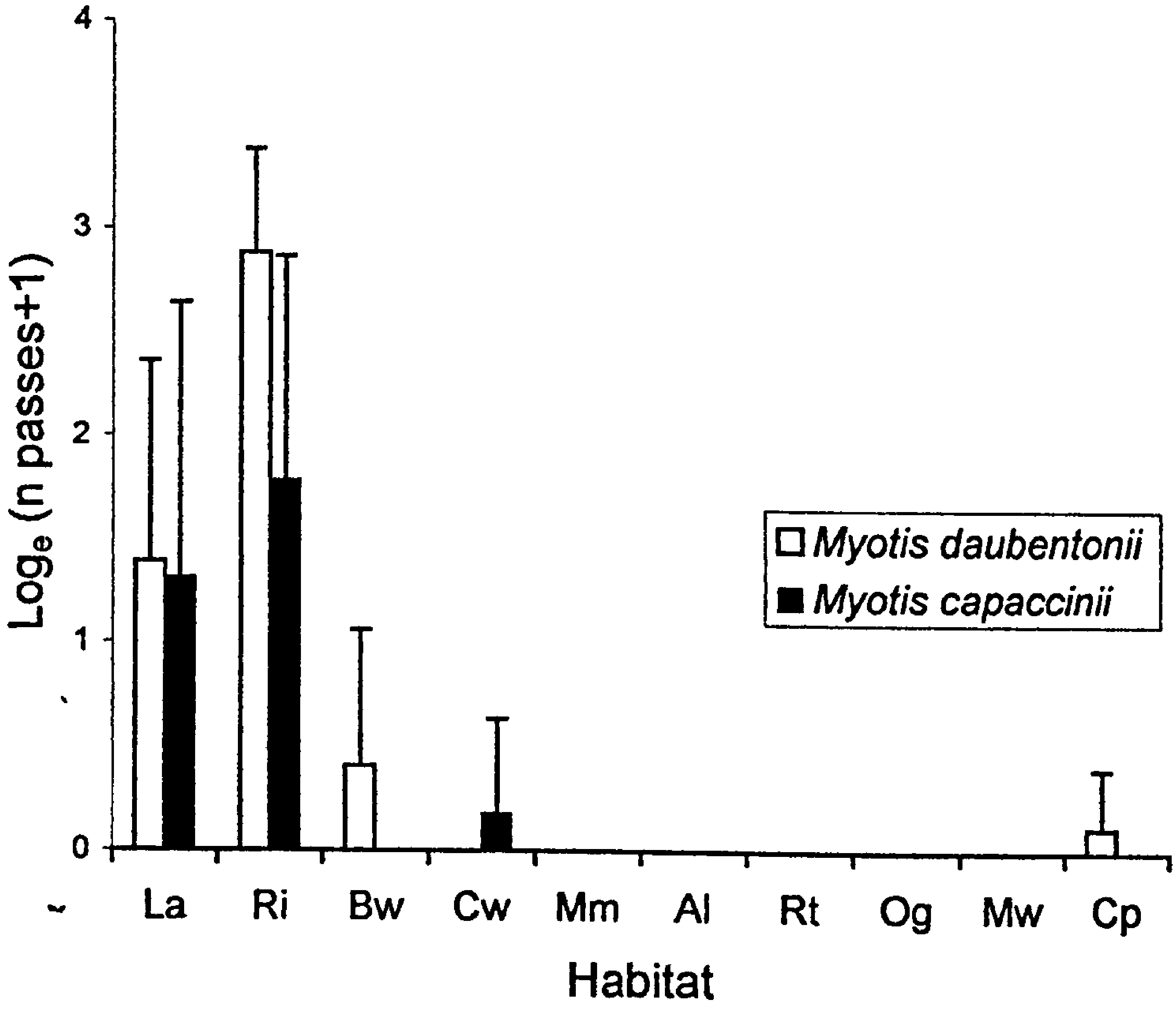

Fig. 6.9. Mean log transformed counts of *Myotis daubentonii* and *M. capaccinii* passes recorded in the 10 habitat types. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

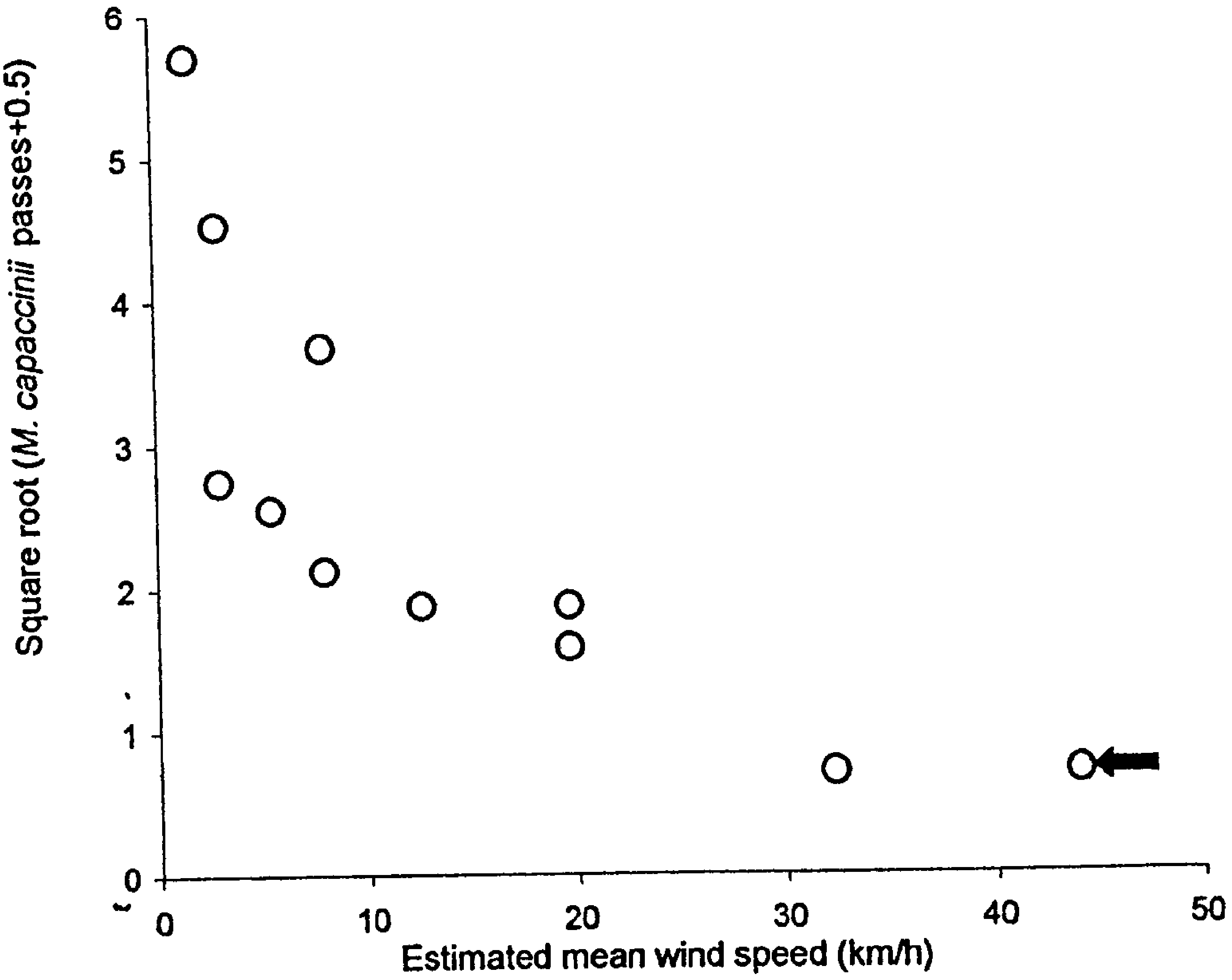

Fig. 6.10. Relationship between square root transformed passes of *Myotis capaccinii* at 12 water sites (6 rivers, 6 lakes) and estimated wind speed ($r_s = -0.95$, $p < 0.001$). Two data points coincide (arrow).

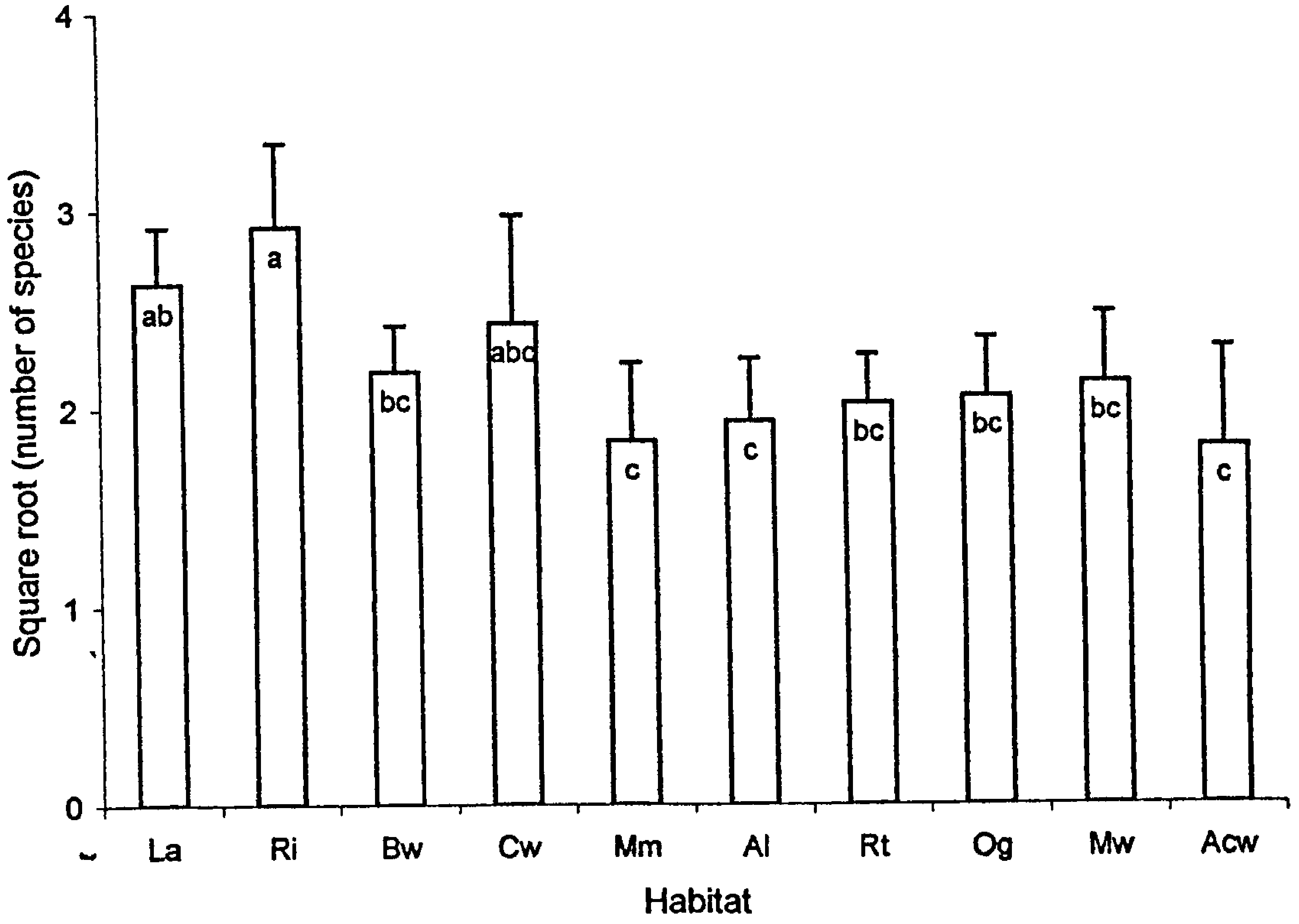

Fig. 6.11. Mean square root-transformed numbers of foraging species detected. La = lakes, Ri = rivers, Bw = beech woodlands, Cw = chestnut woodlands, Mm = Mediterranean macchia, Al = arable land, Rt = rural towns, Og = Olive groves, Mw = Mediterranean and sub-Mediterranean woodlands, Cp = Conifer plantations. Habitats in which no significant difference in bat activity was detected are labelled with the same letter. Standard deviations are shown.

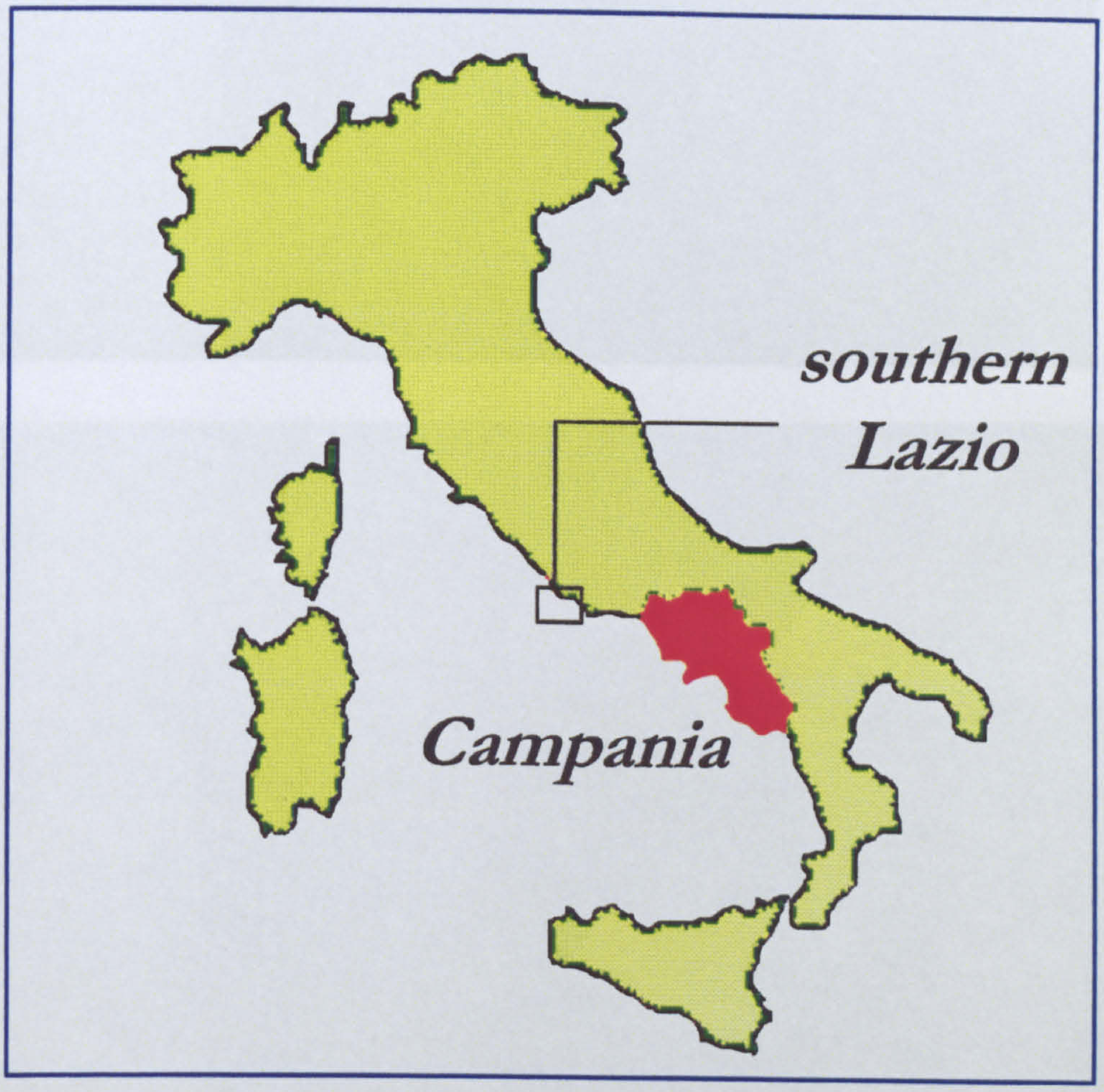

Plate 6.1. Map of Italy showing the location of Campania and coastal southern Lazio, the area of the 60 sites chosen to survey habitat use.

Plate 6.2. San Pietro Lake (above) and Sele river (below), both in Campania *(photograph by S. Viglietti)*.

Plate 6.3. Typical woodlands of *Fagus sylvatica* (above) and *Castanea sativa* (below) *(photograph by S. Viglietti)*.

Plate 6.4. Mediterranean scrubland ('macchia') in a coastal site from Campania (above) and arable land *(photographs by S. Viglietti)*.

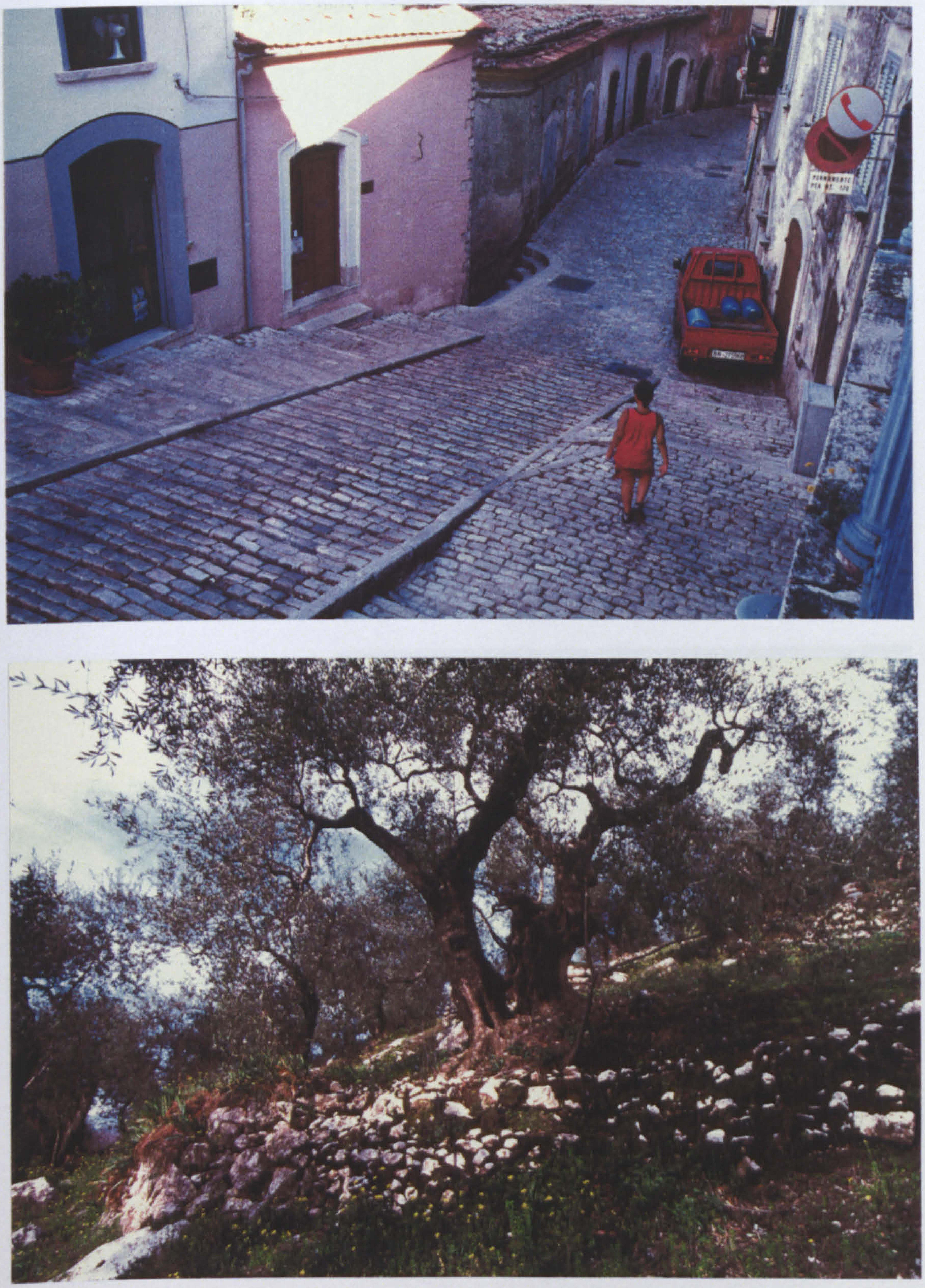

Plate 6.5. Above: the town of Morcone, northern Campania. Below: a typical olive grove *(photographs by S. Viglietti)*.

Plate 6.6. Above: a Mediterranean woodland dominated by *Quercus pubescens*. Below: artificial conifer plantation in the Vesuvius National Park *(photographs by S. Viglietti)*.

6.6. – Appendix I

Quadratic discriminant analysis (with cross-validation) for species emitting FM calls, genera *Myotis*, *Plecotus*, *Barbastella*. Unlike function illustrated in chapter five, the one shown here does not include *M. mystacinus*. Model relied on 4 parameters (start frequency, end frequency, centre frequency, frequency of maximum energy, duration) and provided an overall correct classification rate of 74.2% (n = 360). Random data classification would be 11.1% correct. A MANOVA showed that the model was significant (Wilk's $\lambda = 0.04527$, $F_{32,1284} = 54.156$, $p < 0.001$) and that 82.8% of the variation was explained by the first discriminant function. The first three discriminant functions explained 98.8% of the total variation. Classification rates ranged from 53% (for *M. blythii*) to 88% (for *B. barbastellus*). Classification rates > 70% were obtained for 6 out of 0 species. M. myo. = *M. myotis*, M. bly. = *M. blythii*, M. cap. = *M. capaccinii*, M. ema. = *M. emarginatus*, M. dau. = *M. daubentonii*, M. nat. = *M. nattereri*, Pl. aus. = *P. austriacus*, Pl. aur. = *P. auritus*, B. bar. = *B. barbastellus.*

	True group								
Classified as	M.myo.	M.bly.	M.cap.	M.ema.	M.dau.	M.nat.	Pl.aus.	Pl.aur.	B.bar.
M.myo.	28	12	0	0	0	3	0	0	0
M.bly.	6	26	1	1	3	0	0	0	0
M.cap.	2	1	43	13	3	0	0	0	0
M.ema.	1	1	4	35	1	0	0	0	0
M.dau.	0	7	1	0	48	0	0	0	0
M.nat.	3	0	0	3	0	9	0	0	0
P.aus.	0	0	0	0	0	0	40	2	0
P.aur.	2	2	0	0	0	0	12	20	2
B.bar.	0	0	0	0	0	0	3	4	18
Total *N*	42	49	49	52	55	12	55	26	20
N correct	28	26	43	35	48	9	40	20	18
% correct	67	53	88	67	87	75	73	77	90

Seven. – Habitat selection by the Mediterranean horseshoe bat *Rhinolophus euryale* (Chiroptera: Rhinolophidae) in a rural area of southern Italy and implications for conservation

7.1. – Summary

I studied habitat selection by *Rhinolophus euryale* in a rural area of southern Italy in 1998-2000 by radio-tracking. Two comparisons were carried out, one between habitat occurrence within individual home ranges and within the study area, the other between time spent in each foraging habitat and habitat occurrence within the home range. The first analysis showed that olive groves were over-represented (in comparison with availability) in home-ranges (probably because they were predominantly grouped around the roost) and conifer plantations were under-represented. The second analysis highlighted the importance of woodland for *R. euryale*, while urban, open areas and conifer plantations were avoided. I recommend avoiding the clearing of continuous, large areas of woodland for tree harvesting, and the planting of conifers. Urbanisation should be limited in the areas of greatest importance for the species, and linear landscape elements such as tree lines and hedgerows should be maintained.

Russo, D., Jones, G. and Migliozzi, A. (*in press* a). Habitat selection by the Mediterranean horseshoe bat, *Rhinolophus euryale* (Mammalia: Chiroptera) in a rural area of southern Italy. *Biol. Conserv.*, is based on this chapter.

7.2. – Introduction

The Mediterranean horseshoe bat *Rhinolophus euryale* Blasius, 1853 is one of five rhinolophid species occurring in Europe (Mitchell-Jones *et al.*, 1999). Medium-sized (forearm length = 43-51 mm, body mass = 8-17.5 g; Schober and Grimmberger, 1997), its European range mainly covers the southern part of the continent (Ibáñez, 1999) and particularly the Mediterranean region, as the vernacular name suggests. Although its status is merely classified as 'Vulnerable' in the IUCN Red List (2000) – assessment made in 1996 – data are indeed scarce and populations may be more threatened than this classification indicates: a worrying decline (ca. 70%) occurred in France between 1940 and 1980 and a serious population decrease was also noticed in Slovakia (Brosset *et al.*, 1988; Schober and Grimmberger, 1997; Ibáñez, 1999).

All five European rhinolophids feature in the Italian bat fauna (Lanza, 1959). *Rhinolophus blasii*, however, is probably extinct (Bulgarini *et al.*, 1998), and *Rhinolophus mehelyi* is mainly confined to Sardinia (Mucedda *et al.*, 1994-95). Of the remaining three species, *R. euryale* seems to be the rarest; in fact, although no population estimates are available, it appears to be less common than the greater (*Rhinolophus ferrumequinum*) and the lesser (*Rhinolophus hipposideros*) horseshoe bats (D. Russo, *unpublished data.*).

The species appears to be particularly sensitive to human disturbance, and because it mainly roosts in underground shelters, protection of such sites is undoubtedly a key conservation strategy (Stebbings, 1988; Schober and Grimmberger, 1997; Ibáñez, 1999).

However, besides protecting roosts, bat conservation actions should also address the preservation, correct management and enhancement of foraging sites, because these are fundamental to bats (e.g. Stebbings, 1988; Ransome, 1997; Hutson *et al.*, 2001). Among rhinolophids, in particular, especially valuable information on the selection of foraging habitats has been gathered for *R. ferrumequinum* (Jones and Morton, 1992; Duvergé and Jones, 1994; Jones *et al.*, 1995; Duvergé, 1996; Ransome, 1997; Bontadina *et al.*, 1999a; Duvergé and Jones, *in press*). Such knowledge has been used to implement conservation measures in Great Britain (Ransome, 1997) and to formulate an Action Plan (Ransome and Hutson, 2000). Countryside Stewardship Schemes were also developed to grant-aid farmers for the conservation of habitat features important for this species (Mitchell-Jones, 1998).

Although habitat protection has been emphasised for the conservation of *R. euryale* (Schober and Grimmberger, 1997), the only reports on foraging habitats by this species are largely anecdotal (Schober and Grimmberger, 1997). My project aimed to fill this gap. I studied habitat selection in a *R. euryale* colony from a rural area of southern Italy by radiotracking. I did not apply acoustic survey methods because the chance of detecting this species with a bat detector was low. In fact, like all rhinolophids, *R. euryale* emits high-frequency (Russo *et al.*, *in press* b), highly directional echolocation calls, deeply affected by atmospheric attenuation (e. g. Griffin, 1971; Lawrence and Simmons, 1982). Furthermore, the species was uncommon in the study area and thus unlikely to be encountered on foraging grounds. I paid special attention to the following questions: a) which habitats are most preferred by the species?, and b) can the species adapt to habitats created or substantially modified by man?

The study area appeared well suited to pursuing these goals, because of the occurrence of logging, farming practices, conifer plantations, urbanisation, non-native plant species, alteration of river structural features and water pollution. The landscape in the study area was therefore a rapidly changing mosaic of natural and transformed habitats. I also provide some qualitative observations on commuting, foraging and roosting behaviour of this little known species. The ultimate aim was to collect basic information which may contribute to the development of a conservation plan for *R. euryale*, as recommended by Hutson *et al.* (2001) for bats in the Mediterranean region.

7.3. – Material and methods

7.3.1. – Study area and land use mapping

The study was conducted in June-July 1998-2000 in the Titerno valley (Lat. 41°17'N, Long. 14°30'E) and its surroundings, a rural area of Campania (southern Italy). The Titerno is a stream which largely dries up in summer. The highest elevation of the mountains occurring in the area is 1332 m a.s.l. Hereafter I term 'study area' that (covering 3397.1 ha) delimited *a posteriori* by tracing a Minimum Convex Polygon (MCP; Mohr, 1947) around all locations where the bats tracked were observed (Figs. 7.1-7.4).

The following habitat types were recognised by field surveys and analysis of aerial photographs:

1) Broadleaved woodland (Plate 7.1). This was mainly constituted by evergreen *Quercus ilex* coppice in some areas, in others by a complex association of several tree species, i.e. *Quercus pubescens*, *Alnus cordata*, *Ostrya carpinifolia*, *Castanea sativa* and frequent aliens such as *Robinia pseudoacacia* and *Ailanthus altissima*. Major tree hedgerows were included.
2) Riparian woodland (Plate 7.2). This occurred along the shores of the Titerno stream and the Volturno river (a limited part of the latter was comprised within the west sector of the study area). Riparian woodland bordering the Titerno had been altered considerably (Plate 7.3), and in several riparian areas native tree species such as willows and poplars had been substituted by invasive arboreal species such as *R. pseudoacacia* and *A. altissima*. Large strips of woodland bordering canals in farmland (a poorly represented habitat in the study area), of species composition similar to that of altered riparian vegetation in the study area, were also included in this category.
3) Riparian scrubland (Plate 7.4). Riparian low vegetation, dominated by shrubs and herbaceous species, with rare trees.
4) Scrubland, grassland and clearings (Plate 7.4). This category included open sites, i.e. sites with shrubs (excluding riparian scrublands), grass and bare ground. Clearings produced by logging and fire were included.
5) Olive groves (Plate 7.5). These were generally patchy and interspersed with small woodland tracts, constituting a complex mosaic. Undergrowth often occurred in olive groves.
6) Farmland (Plate 7.5). Arable land, including fields with herbaceous cultivations, vineyard, small and isolated olive groves and orchards. Such varied cultivations often constituted a complex mosaic.
7) Conifer plantation. Planted *Pinus* spp. woodland.
8) Urban (Plate 7.6). Small towns; patchy built-up sites surrounded by farmland.

A land use map was generated with the GIS software Arcview 3.1 (ESRI inc.). Mapping was accomplished by photo interpretation of 1:10.000 orthophotos (AIMA, Italy). Screen digitisation of photographs was integrated and corrected with data from field surveys. Topology was verified with Ilwis 2.23 (ITC© The Netherlands). The map was referred to the Gauss-Boaga coordinate system projection for Italy (Zone 2).

7.3.2. – The colony

The bats roosted in an artificial underground shelter (Plate 7.7) about 1-km long located at an elevation of ca. 200 m a. s. l. and comprising four different branches. These were characterised by different ambient temperatures: the branch used in summer by *R. euryale* was the warmest, its mean winter temperature being ca. 12°C (about 3°C higher than that measured in the others; D. Russo, *unpublished data*). Although temperature was not measured in summer, on occasional summer visits I noticed that the thermal difference clearly persisted, and perhaps even increased because of the heat produced by the larger congregation of bats.

In summer, *R. euryale* roosted together with *Miniopterus schreibersii* (Plate 7.8), *Myotis emarginatus*, *Myotis capaccinii* and a few non-breeding *R. ferrumequinum*. In summer 1997, the overall colony size was estimated to be ca. 500 bats, about 200 of which were *R. euryale.* Although counts were not carried out on a regular basis, occasional observations on emergence and repeated captures in order to tag bats showed a large seasonal fluctuation in the number of individuals of all species frequenting the roost. This varied from a few hundred in May to only 20-40 from mid-July to the beginning of August. Bats probably moved to different sites soon after breeding, as some of my results suggested (see below). The reproduction of *R. euryale* was ascertained in all three years of study. It was not clear whether the other species (apart from the non-breeding *R. ferrumequinum*) regularly bred at the site over the study period. *R. euryale* newborns and females in late pregnancy were observed from mid-June to mid-July.

7.3.3. – Tagging procedure and data collection

My methods followed those used for similar studies on the closely related *R. ferrumequinum* (Jones and Morton, 1992; Jones *et al.*, 1995; Duvergé, 1996). Bats were captured while leaving the roost with a harp-trap and fitted with a Holohil radio-transmitter, attached between the shoulder blades with Skinbond® adhesive after clipping the fur (Plate 7.9). In 1998, I used Holohil (Carp, Canada) BD-2 0.72 g tags. Although these only slightly exceeded 5% of body mass (Aldridge and Brigham, 1988) – a condition that did not affect bats in other radio-telemetry studies (e.g. Entwistle *et al.*, 1996; Sedgeley and O'Donnell, 1999) – in order to avoid all risk of influencing the bats, in the following years Holohil LB-2 tags weighing 0.5g (Tab. 7.1; Plate 1.3) were used.

A Lotek (Newmarket, Canada) Suretrack STR1000 receiver connected to a three-element Yagi antenna was used in the field (Plate 1.3). Each night, a certain individual was tracked (focal subject) and the presence of others was checked repeatedly. Whenever contact was lost with the focal subject for more than ca. 15 minutes, another bat in contact was followed.

Continuous tracking was adopted (e.g. Jones and Morton, 1992; Duvergé 1996): once a bat was detected, several bearings were taken in rapid sequence. To locate a bat, the 'homing in' method was applied (e.g. White and Garrott, 1990; Entwistle *et al.*, 1996): the observer established the bat's position by approaching the subject tracked as close as possible by car or on foot. Care was taken not to interfere with the bat's activity. Variations in direction and intensity of radio-signals were interpreted to find out whether a bat was actually foraging or commuting. Signals that were very strong and almost or completely non-directional – even when the receiver gain was lowered to 'zero'– were attributed to close proximity of the bat tracked. This assumption was shown to be correct as such signal characteristics were also noticed when emerging bats were detected by an observer located by the roost exit, and on one occasion when a tracked bat was seen foraging a few metres from the receiver. Interpretation of radio-signals was also tested in the field by activating a tag and placing it in various locations within the study area. The bat's position (hereafter termed fix; Tab. 7.1) was noted on a 1:25.000 map (Istituto Geografico Militare, Firenze) and the activity performed by the bat was recorded. Rapid, directional movements between distant sites were classified as commuting; the behaviour of a bat which kept flying in a defined area of variable size (i.e. a foraging area) was interpreted as foraging. Foraging and commuting times were recorded; when activity was unclear, the corresponding time was attributed to an 'undetermined behaviour' category, and not considered for analysis. On rare occasions, in woodland, a bat appeared completely motionless for >20 min. Although the bat could not be seen, it was likely to be night-roosting hanging from a tree (see 'Results'), and such a period was excluded from foraging time budget and not used for analysis.

In 1998, field work was limited by the impossibility of receiving signals from bats within the roost. To solve this problem, two aerials fitted with about 200m of BNC cable were set up within the roost. Sometimes the aerials made it possible to verify the presence of the bats within the roost.

7.3.4. – Data analysis

A point vector map of all bat positions was obtained with Ilwis, and fixes were associated to a database including subject code, activity performed, time of observation (start – end) and duration of the behaviour observed (in minutes).

Minimum Convex Polygons (MCPs) delimiting respectively all fixes (i.e. the study area) and those corresponding to each bat (individual home ranges) were digitised with Arcview, and percent habitat composition within MCPs was calculated. Habitats corresponding to each bat location were determined through overlay operations carried out with the Arcview 3.1 geo-processing extension (Spatial Join). Point and land use maps were rasterised to perform overlay procedures. The time spent by each bat in each habitat type was then calculated. Two different analyses comparing habitat use with habitat availability were performed:

a) Percent habitat composition within each bat's MCP was compared with that of the study area to determine whether habitat occurrence within individual home-ranges was non-random (i.e. differing significantly from that of the study area).
b) Percent foraging time spent by bats in each habitat was compared with percent habitat proportion occurring within individual home-ranges to see whether the bats selected habitats or used them according to availability.

Hence while analysis (a) concerned all observations (i.e. regardless of whether they corresponded to foraging or other behaviours), (b) focused on foraging time and foraging sites. An MCP including all bat positions to define habitat availability was adopted because this was independent of the roost position. In fact, a few days after tagging, several bats moved to different roosts (apparently located in the same general area) only one of which could be exactly located – some of these roosts may have been underground shelters, from which radio-signals could not be detected. The reason for this behaviour may have been that the bats – mostly lactating females – left the nursery soon after weaning the young. In this situation, it was impossible to define the area potentially available to the bats as a circle around the roost with a radius equal to the maximum distance travelled from the roost (maximum range circle, MRC) as done in other studies (e.g. Waters *et al.*, 1999). In this paper, I call 'main roost' the one where all study subjects were captured.

In 1999 and 2000, two adjoining woodland areas of ca. 12 ha each were cleared (one in each year). The resulting clearings were classified as 'scrubland, grassland and clearings'(Plate 7.9). The proportions of woodland and 'scrubland, grassland and clearings' occurring in the study area in the corresponding years were therefore corrected for, and adjustments were made on habitat percentages relative to one individual MCP whose area partly included the cleared site.

Both comparisons were carried out using Compositional Analysis (Aebischer *et al.*, 1993). In this method, log-ratio differences between proportions of used and available habitats are entered for analysis, and each bat represents a sample unit (Aebischer *et al.*, 1993). The analysis involves two steps: first, a multivariate test for non-random habitat use is performed; then, if results show actual selection to occur, pairwise comparisons between habitats are made to rank habitat types in a decreasing order of importance.

To overcome problems determined by departure from multivariate normality of log-ratio difference distribution, I calculated the significance of Wilk's Λ and t statistics by randomisation tests as recommended by Aebischer *et al.* (1993). When availability was zero, the missing values in a given residual log-ratio were replaced by the mean of all non-missing values for that log-ratio. A mean Λ was then obtained as a weighted mean (Aebischer *et al.*, 1993), and 1000 iterations were employed in randomisation tests (Manly, 1997). Where proportions of 'used' were zero, these were substituted by 0.001, a figure which is a magnitude less than the smallest value occurring in the data set (Aebischer *et al.*, 1993).

Habitat ranking matrices were obtained, showing the significance levels relative to pairwise comparisons between habitat types obtained from randomisation tests. In the matrices, I indicate whether the habitat placed in the corresponding row was preferred more (+) or less (-) than that in the corresponding column. A triple sign (+++, —) was used to indicate occurrence of significant differences, one sign shows non-significant trends.

Habitats were then ranked according to their relative importance, each rank being determined by the number of (+), (+++) signs occurring in the corresponding row. A simplified rank order was so obtained, habitats before '>' symbols being preferred to those following the sign. The symbol >>> separates habitats which differed significantly, while > is placed between those which did not. Analyses were performed with the software Compositional Analysis Excel tool 3.1 written by Peter Smith (University of Aberdeen). All statistics, matrices and rank orders were provided automatically by this software.

The bats could rarely be tracked continuously over most or all of one night. Possible reasons for frequent interruptions in signal reception were landscape complexity (presence of obstacles such as mountains and canyons which obscured the signal), the bats' low flight (as noticed on occasional sights of the subjects tracked) and maybe the use of underground night roosts (potential cave roosts were largely available in the area) from which radio signals could not be detected. Apart from the main roost, only one of what were probably several roosts was identified. This made it difficult to compute an average value for the maximum distance travelled from the roost each night. This problem did not affect my primary goal, i.e. to determine selection of foraging habitats. Nevertheless, I determined with Arcview the furthest distance travelled by the bats on nights when they left a known roost. The value range obtained may be regarded as a descriptor of maximum nightly movement from roost of the bats tracked. I used Ilwis to determine the highest elevations of the sites frequented by the bats by overlaying all bat locations with a digital elevation model (DEM) calculated by point interpolation.

7.4. – Results

7.4.1. – Habitat selection

Eighteen adult bats were tagged, five (all females) in 1998, seven (five females, two males) in 1999 and six (four females, two males) in 2000 (Tab. 7.1). All females were lactating or in early post-lactation when they were captured. A female tagged in 1998 (C; Tab.7.1) was tracked for less than 40 minutes, so those data were not included in the analysis, and a male tagged in June 2000 was never detected after release. Data from 16 bats were used to determine habitat selection. Data collection ended when contact with the bats was lost in all cases except two, when the tag fell off.

Percent habitat composition of individual MCP areas (used; Tab. 7.2, Figs. 7.1 – 7.4), compared with that of the study area (available; Tab. 7.3, Figs. 7.1 – 7.4) was non-random (weighted mean Wilk's $\Lambda = 0.1504$, $\chi^2 = 30.3076$, d. f. = 7 $p < 0.0001$, randomisation $p < 0.008$). Comparison between habitats (Tab. 7.4) provided the following simplified rank order: olive groves >>> broadleaved woodland > urban > riparian scrubland > riparian woodland > scrubland, grassland and clearings > farmland >>> conifer plantation.

The comparison between time spent foraging by bats in each habitat and habitat proportions occurring in their MCPs (Tabs. 7.2, 7.5, Figs. 7.1, 7.2, 7.3, 7.4) provided more information: a larger number of significant differences between habitats emerged. Again, habitat use departed significantly from random when compared with habitat availability (weighted mean Wilk's $\Lambda = 0.0044$, $\chi^2 = 86.6700$, d. f. = 7 $p < 0.0001$, randomisation $p < 0.001$). The rank order (Tab. 7.6) was: broadleaved woodland >>> olive groves > riparian woodland > riparian scrubland > farmland >>> conifer plantation > scrubland, grassland and clearings >>> urban. Broadleaved woodland emerged as the preferred habitat; conifer plantation, scrubland-grassland and clearings, and urban were least preferred – in these habitats no bat was ever observed foraging (Tab. 7.5). All bats observed foraging in farmland actually used tree lines and hedgerows rather than open fields.

7.4.2. – Activity patterns and behaviour observed

Maximum distance travelled from the roost on one night by a bat (n = 14) averaged about 2.2 km (Tab. 7.1); the furthest recorded was about 5 km. Maximum elevation of sites frequented by individual bats (n = 17) ranged between 236-580 m a. s. l.(Tab. 7.1). In this study, it was clear that bats followed the stretches of riparian vegetation bordering the dried up stream as a navigation landmark (Fig. 7.5), besides using them to forage. This behavioural pattern was observed in 9 out of 16 bats. One of the bats tracked (bat 'R') reached a previously unknown roost (Plate 7.9) 8.4 km away from the main one. On its way to the roost, bat 'R' crossed several kilometres of farmland following large hedgerows and woodland strips. Although these landscape elements were also used to forage (and probably to night-roost), they clearly served as navigation landmarks.

The roost used by bat 'R' was a bridge over a river (Plate 7.9): the bats used a space which was in part occupied by a large water conduct. Because water percolated from the conduct, the roost was very humid besides being dark, and this determined suitable roosting conditions. The roost was used by *R. euryale*, *M. schreibersii*, *M. capaccinii* and *M. emarginatus* (i.e. the same species association found in the main roost).

Direct observation was difficult because the bats mostly selected wooded sites. Only one bat was seen foraging, on the wing. In that case, the signal varied rapidly in direction and intensity within the foraging site, as was often noticed for foraging bats tracked but not observed. Furthermore, another typical signal variation was associated with bats foraging in wooded sites: rapid changes in signal strength and direction within a very limited woodland spot (i.e. very short-range movements) alternating with a stationary condition. Although the bats were not seen, in such cases they may have been perch-feeding.

I had strong evidence that bats night-roosted in trees although I could not observe this behaviour directly. Some bats stayed completely immobile in a woodland site – at least judging from the signal features – for durations of up to 78 minutes. On one night, bat 'R' stopped hunting in a riparian woodland site and night-roosted for 177 minutes hanging from the same bridge where its day roost was located (roost entrance was ca. 30 m from the night-roosting spot).

7.5. – Discussion

7.5.1. – Comparison of habitat composition between individual MCPs and study area

The two comparisons performed to determine habitat selection by *R. euryale* showed me discrepancies in habitat ranking according to habitat relative importance. The comparison between habitat occurrence in MCPs and in the study area showed that olive grove was the most preferred habitat. This may be due to the fact that olive groves were mostly concentrated around the main roost (Figs. 7.1-7.4). The area around the roost was clearly included in all home ranges, and this emphasized the 'weight' of olive groves in the analysis. Likewise, a significant proportion of urban habitat featured in most individual MCPs because one of the few considerable urban sites in the study area was close to the roost (Figs. 7.1 – 7.4): therefore this habitat obtained the third highest rank. The negative selection that emerged for conifer plantation had a stronger ecological significance. This habitat mainly occurred not far from the roost (see sites shown in black in Figs. 7.1 – 7.4) and could therefore easily be reached by the bats. In spite of this, conifer woodland was clearly avoided. Unlike conifer woodland, broadleaved woodland was selected positively: it was the second habitat in order of importance. Although these results do show some habitat choice, it appears that the analysis based on individual MCP habitat composition is not sufficiently sensitive to investigate habitat selection by this species in detail. Indeed, the bats often commuted within the home range to reach favourable, and quite often small, hunting sites located far apart where most foraging occurred. The highly patchy landscape may have exaggerated this behaviour: suitable foraging sites were scattered over home ranges. For example, among the preferred hunting sites were several narrow wooded canyons surrounded by large olive groves on the flank of a mountain. Despite their limited size, these canyons were used frequently and foraging time spent there had an important effect on the final time budget. The same considerations apply to fragments of broadleaved woodland in farmland and to restricted riparian woodland spots.

7.5.2. – Comparison between percent foraging time in each habitat and individual MCP habitat composition

The analysis of foraging time provided a more effective picture of habitat selection. Two woodland types – broadleaved and conifer – were respectively the most and one of the least preferred habitats (none of the tracked bats ever foraged in conifer woodland). The use of wooded strips as navigation landmarks and the probable tree night-roosting behaviour further highlight the importance of broadleaved woodland for *R. euryale*.

Analysis of foraging time also showed that the species had a preference for olive groves. Olive groves in the study area were managed in a traditional and not intensive way: they often had some undergrowth and were interspersed with woodland patches. Moreover, they were generally adjacent to major woodland areas. Their structure may therefore have been sufficiently close to that of a 'natural' woodland to be utilised for foraging. Why riparian woodland was less important than broadleaved woodland found elsewhere is unclear. The large, frequent gaps occurring in this often degraded habitat might contribute to diminish their importance in the study area. When bats were observed foraging in farmland, in all cases they used wooded hedgerows and tree lines: the occurrence of these structures in arable land should therefore be maintained and enhanced. Scrublands (apart from the riparian ones) and other open vegetation sites such as grassland and clearings were avoided.

Finally, urban areas were the least preferred habitat in the classification obtained by comparing foraging time vs. individual MCP habitat composition. This was not evident in the individual MCPs vs. study area analysis because the largest built-up area (a town) again occurred close to the roost and to some major foraging sites, and was therefore over-represented in the results. Indeed, no bat was ever observed foraging or even crossing built-up areas when commuting: bats clearly detoured to avoid urban sites when commuting between roost and foraging grounds.

Studies on the closely related *R. hipposideros* (Schofield, 1996; Bontadina et al., 1999b) in Wales led to results very similar to ours. Like *R. euryale*, *R. hipposideros* mainly selected broadleaved woodland, riparian vegetation, hedgerows and tree lines, whilst conifer plantations, low vegetation, settlements and bare areas were avoided. Hedgerows, tree lines and stretches of riparian vegetation were used as landmarks.

Conifer plantations have been found to be avoided by other bat species which select broadleaved woodlands for foraging, such as *Plecotus auritus* (Entwistle et al., 1996) and *Myotis nattereri* (Smith, 2000). Acoustic surveys (chapter six) showed that in southern Italy conifer plantations were avoided by another bat species, *Hypsugo savii*, and that a rather low number of species foraged in this habitat.

The probable reason for avoiding conifer woodlands is that they support fewer prey-insects than broadleaved ones (Waring, 1988; 1989; Entwistle et al., 1996), including Lepidoptera – an important component in the diet of *R. euryale* (Koselj and Krystufek, 1999).

The presence of woodland is also important for *R. ferrumequinum*, whose preferred foraging habitats are grazed pastures interspersed with blocks or strips of deciduous woodland and significant hedgerows (e.g. Duvergé, 1996; Ransome and Hutson, 2000).

7.5.3. – Implications in habitat protection and management

My results have clear implications for habitat management. Reforestation with non-native conifers – a forestry practice widely applied until recently in Italy, and not yet abandoned – is harmful to *R. euryale* (and probably to several other bat species) and should be discouraged; native broadleaved tree species should be planted instead.

The logging which occurred in the study area in 1999-2000 cleared large, continuous woodland areas (Plate 7.9): not only did this reduce the availability of a preferred foraging habitat (woodland), but it also increased the occurrence of a very unfavourable one (clearings). My results suggest that an especially careful logging protocol should be applied in managing coppice within areas of major importance for *R. euryale*: the logging of large areas should be avoided, minimising the size of single clearings. In this way, a system of woodland corridors and patches (providing commuting landmarks) connecting the areas of undisturbed woodland is created. Grindal and Brigham (1998) showed that small forest openings may lead to an increase in bat activity. As for many bat species (e.g. Krull *et al.*, 1991; Limpens and Kapteyn, 1991; Duvergé, 1996; Sierra-Cobo *et al.*, 2000), navigation landmarks are important for *R. euryale*. In my study, these coincided with foraging areas and were therefore important for both hunting and commuting behaviour. Riparian vegetation, hedgerows and tree lines should therefore be scrupulously protected and encouraged.

Urbanisation is a major threat to *R. euryale*, which avoided urban areas. Although some more adaptable bat species may take advantage of roosting and foraging opportunities offered by urban areas (e.g. Kunz, 1982), in general urbanisation is harmful to insectivorous bat communities (Kurta and Teramino, 1992; Gaisler *et al.*,1998).

The elevation values where roosts and foraging sites were located, lower by far than the highest occurring in the study area, confirm the tendency of this species to roost and hunt at a low altitude (Schober and Grimmberger, 1997; Ibáñez, 1999). This should be taken into account in the identification of areas for special conservation efforts.

My data on distances between roost and foraging areas should be considered as preliminary and treated with prudence until further, more complete information on the commuting range of *R. euryale* is gathered. However, they are similar to those obtained in several studies on other rhinolophids (Jones and Morton, 1992; Duvergé and Jones, 1994; Bontadina *et al.*, 1999b). A conservative approach would require protection of foraging habitats within a range of not less than 5 km from nurseries.

Tab. 7.1. Date of capture, sex, biometry (FAL = forearm length, BM = body mass), percent ratio tag/body mass and tracking details of the bats studied. Days tracked include all nights when at least one contact with the bat was obtained. M = male, F = female. Tags used in 1998 weighed 0.72 g, all others 0.50 g. Max distance and Max elevation are the maximum distance flown from the roost and the highest elevation reached by each bat. Distance for bat 'R' was measured from the second roost discovered during the study.

Bat code	Date of capture	Sex	FAL (mm)	BM (g)	100x (tag mass/BM)	Days tracked	n fixes	Time tracked (min)	Max distance (m)	Max elevation (m)
A	05 July 98	F	47.9	10.7	6.7	4	41	221	4618	580
B	05 July 98	F	48.2	11.2	6.4	4	33	406	4156	393
C	17 July 98	F	49.4	11.2	6.4	3	39	255	1946	344
D	17 July 98	F	48.5	11.2	6.4	3	41	238	865	368
E	05 July 98	F	48.2	11.1	6.5	2	4	38	-	236
F	05 July 99	F	49.0	11.1	4.5	8	59	471	1840	525
G	05 July 99	F	47.0	10.5	4.8	4	31	156	2077	382
H	05 July 99	F	47.0	11.2	4.5	3	21	301	-	408
I	05 July 99	F	47.8	11.2	4.5	3	23	155	2503	320
J	15 July 99	M	48.5	11.1	4.5	5	87	927	1129	492
K	15 July 99	M	45.2	10.7	4.7	3	21	137	446	565
L	15 July 99	F	49.7	13.8	3.6	4	34	201	-	290
M	27 June 00	F	48.9	10.8	4.6	6	68	445	2656	393
N	27 June 00	F	48.5	10.6	4.7	3	22	87	694	323
O	27 June 00	F	48.5	10.6	4.7	3	27	142	879	272
P	10 July 00	F	47.7	10.5	4.8	7	61	345	2253	456
Q	27 June 00	M	48.4	11.4	4.4	-	-	-	-	-
R	13 July 00	M	47.5	10.3	4.8	6	52	1120	5035	300
	Mean (S.D.)		48.1 (1.0)	11.1 (0.8)	5.1 (0.9)	4.2 (1.7)	39.1 (20.8)	332 (289.5)	2221 (1474)	391 (102.4)

Tab. 7.2. Percent habitat composition of MCPs of 16 *R. euryale*.

Bat	MCP area (ha)	Broadleaved Woodland	Riparian woodland	Riparian scrubland	Scrubland, grassland and clearings	Olive groves	Farmland	Conifer plantations	Urban
A	936.8	19.23	1.65	6.76	5.29	29.43	30.06	4.41	3.17
B	215.4	12.80	0	2.97	2.20	68.98	11.70	0.89	0.46
C	126.0	38.90	9.41	2.84	0.06	18.66	28.57	0	1.56
D	102.6	14.83	9.41	2.15	0.45	52.38	12.20	0	8.58
F	345.4	21.04	4.07	1.84	4.26	48.27	13.36	0	7.16
G	333.8	18.87	5.68	2.71	0.37	36.85	28.25	0	7.27
H	33.9	11.26	0	0	0	88.74	0	0	0
I	418.9	17.34	6.73	3.03	0.44	21.01	43.60	0.60	7.25
J	439.6	20.83	4.91	1.70	0.59	37.86	27.07	0	7.04
K	54.5	14.55	0	0	6.50	78.95	0	0	0
L	163.0	48.27	11.74	2.97	10.14	5.55	14.86	3.86	2.61
M	1229.4	17.98	3.77	4.57	5.27	24.38	39.95	0.85	3.23
N	99.9	13.62	17.34	3.18	0.24	40.66	16.44	0.01	8.51
O	350.5	7.12	1.17	13.59	0.06	42.30	31.97	0.10	3.69
P	504.0	14.76	3.69	1.57	1.11	33.09	39.10	0	6.68
R	1281.3	10.53	4.74	0.24	0.08	9.23	71.23	0	3.95
Mean	414.7	18.87	5.27	3.13	2.32	39.77	25.52	0.67	4.45
(S.D.)	(397.4)	(10.52)	(4.79)	(3.27)	(3.06)	(23.56)	(18.08)	(1.39)	(3.05)

Tab. 7.3. Size and percent habitat composition of the study area (determined as the minimum convex polygon including all bat positions recorded over 1998-2000). In 1999 and 2000, woodland decreased ca 1.6% each year, and clearings (included in scrubland, grassland and clearings) increased accordingly.

Year	Total area (ha)	Broadleaved woodland	Riparian woodland	Riparian scrubland	Scrubland, grassland and clearings	Olive grove	Farmland	Conifer plantation	Urban
1998	3397.1	21.75	3.25	2.43	3.23	13.85	51.33	1.55	2.61
1999	3397.1	21.39	3.25	2.43	3.59	13.85	51.33	1.55	2.61
2000	3397.1	21.04	3.25	2.43	3.94	13.85	51.33	1.55	2.61

Tab. 7.4. Ranking matrix for *R. euryale* based on comparing proportions of habitats occurring within individual MCPs and those in the study area. Three signs are given for significant deviation from random at $p < 0.05$.

	Broadleaved woodland	Riparian woodland	Riparian scrubland	Scrubland, grassland and clearings	Olive grove	Farmland	Conifer plantation	Urban	Rank
Broadleaved woodland		+	+	+++	---	+++	+++	+	6
Riparian woodland	-		-	+	-	+	+++	-	3
Riparian scrubland	-	+		+	---	+++	+++	-	4
Scrubland, grassland and clearings	---	-	-		---	+	+++	-	2
Olive grove	+++	+	+++	+++		+++	+++	+	7
Farmland	---	-	---	-	---		+++	---	1
Conifer plantation	---	---	---	---	---	---		---	0
Urban	-	+	+	+	-	+++	+++		5

Tab. 7.5. Overall foraging time (minutes) and percent foraging time per habitat for 16 *R. euryale*.

Bat	Foraging time	Broadleaved woodland	Riparian woodland	Riparian scrubland	Scrubland, grassland and clearings	Olive groves	Farmland	Conifer plantations	Urban
A	186	73.12	0	0	0	18.82	8.06	0	0
B	219	85.84	0	2.74	0	11.42	0	0	0
C	223	33.19	30.49	2.24	0	27.35	6.73	0	0
D	196	58.17	12.24	3.06	0	26.53	0	0	0
F	137	83.08	1.99	0	0	14.43	0.50	0	0
G	120	29.17	0	0	0	38.33	32.5	0	0
H	266	57.14	0	0	0	42.86	0	0	0
I	102	32.05	2.91	0	0	15.53	49.51	0	0
J	807	64.13	3.94	0	0	19.43	12.50	0	0
K	122	61.48	0	0	0	38.52	0	0	0
L	186	15.59	74.73	4.84	0	3.23	1.61	0	0
M	342	61.11	10.82	2.05	0	25.15	0.87	0	0
N	80	63.75	17.50	3.75	0	7.50	7.50	0	0
O	101	70.30	0	12.87	0	11.88	4.95	0	0
P	282	38.66	18.09	2.13	0.35	40.42	0.35	0	0
R	474	23.21	70.04	0	0	6.75	0	0	0

Tab. 7.6. Ranking matrix for *R. euryale* based on comparing percentage of time spent foraging in each habitat with the proportions of habitats occurring within individual MCPs. Three signs are given for significant deviation from random at $p < 0.05$.

	Broadleaved woodland	Riparian woodland	Riparian scrubland	Scrubland, grassland and clearings	Olive grove	Farmland	Conifer plantation	Urban	Rank
Broadleaved woodland		+	+++	+++	+++	+++	+++	+++	7
Riparian woodland	-		+	+++	-	+	+	+++	5
Riparian scrubland	---	-		+++	---	+	+++	+++	4
Scrubland, grassland and clearings	---	---	---		---	-	-	+++	1
Olive grove	---	+	+++	+++		+++	+++	+++	6
Farmland	---	-	-	+	---		+++	+++	3
Conifer plantation	---	-	---	+	---	---		+	2
Urban	---	---	---	---	---	---	-		0

Fig. 7.1. Individual home ranges (Minimum Convex Polygons, MCP) of bats A, B, C, D tracked in 1998 (letters correspond to bats as in Tab. 7.1). The study area is delimited by a MCP enclosing all locations of the bats followed during the entire study.

Fig. 7.2. Individual home ranges (Minimum Convex Polygons, MCP) of 4 bats (F, H, I, L) tracked in 1999. Letters correspond to bats as in Tab. 7.1. The study area is delimited by a MCP enclosing all locations of the bats followed during the entire study.

Fig. 7.3. Individual home ranges (Minimum Convex Polygons, MCP) of 3 bats (G, J, K) tracked in 1999. Letters correspond to bats as in Tab. 7.1. The study area is delimited by a MCP enclosing all locations of the bats followed during the entire study.

Fig. 7.4. Individual home ranges (Minimum Convex Polygons, MCP) of bats M, N, O, P, R tracked in 2000. Letters correspond to bats as in Tab. 7.1. The study area is delimited by a MCP enclosing all locations of the bats followed during the entire study.

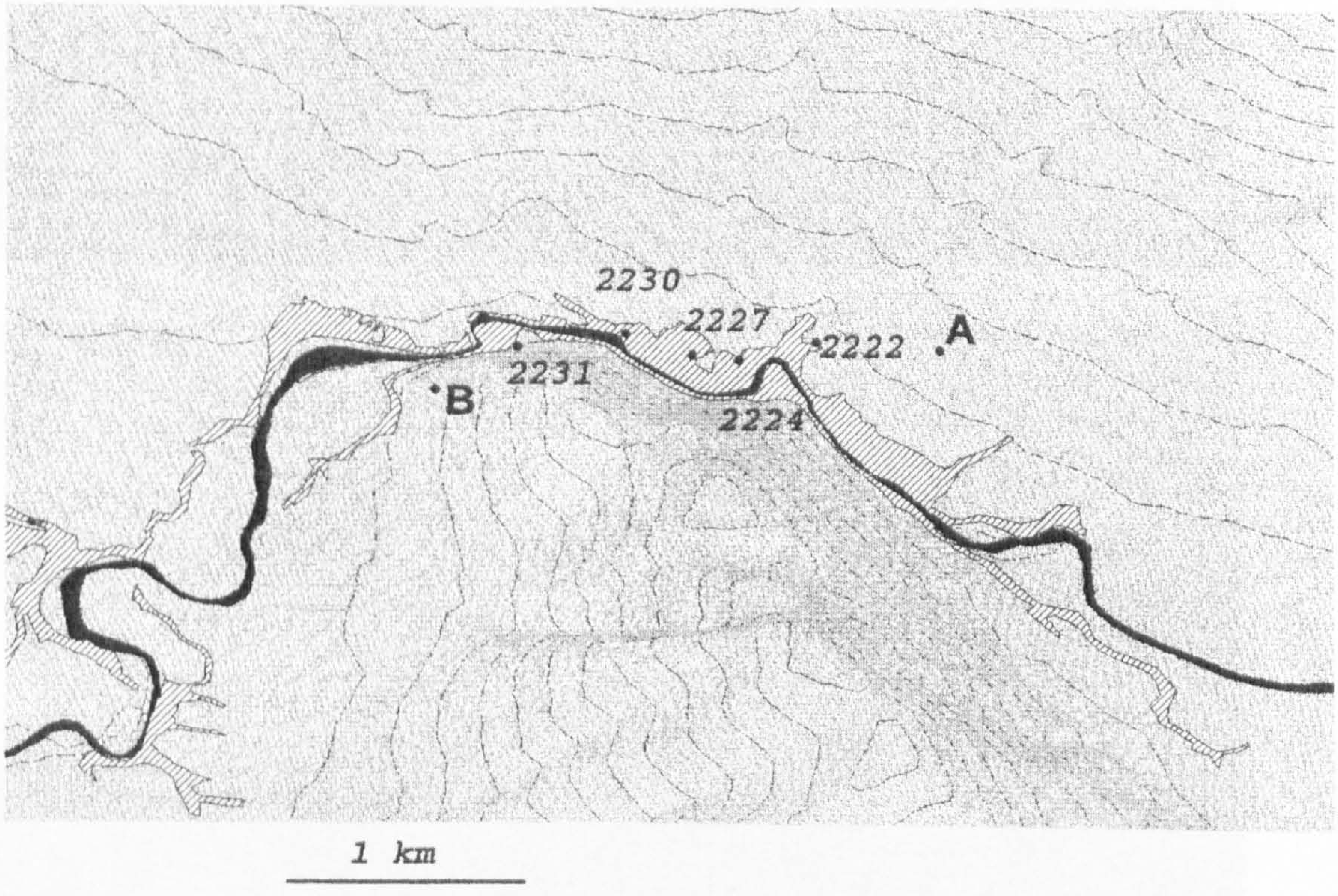

Fig. 7.5. A typical navigation pattern of *R. euryale* following the stream vegetation. On 16 July 1999, bat J left the olive grove foraging site 'A' (2220) and moved west to a woodland foraging site ('B'), which was reached after 15 minutes. Circles illustrate consecutive bat locations; the corresponding time (local time) is also shown. Riparian scrubland is marked in black; dashed areas correspond to riparian woodland.

Plate 7.1. Aspects of the broadleaved woodland in the radiotracking area. A small olive grove surrounded by woodland may be noticed below *(photographs by G. Mastrobuoni)*.

Plate 7.2. Riparian woodland along the dry Titerno stream (above) and (below) the Volturno river *(photographs by G. Mastrobuoni)*.

Plate 7.3. The Titerno stream is profoundly altered. Above: concrete channelisation structure. Below: a rubbish dump along the shores of the stream *(photographs by G. Mastrobuoni)*.

Plate 7.4. Above: clearings and broadleaved woodland on the flank of Monte Monaco di Gioia, the highest peak in the area. Below: riparian scrubland along the Titerno stream *(photographs by G. Mastrobuoni)*.

Plate 7.5. Olive grove (above) and farmland (below) in the radiotracking study area. *(photographs by G. Mastrobuoni)*

Plate 7.6. Two views of the town of Faicchio, the largest human settlement in the radiotracking study area *(photographs by G. Mastrobuoni)*.

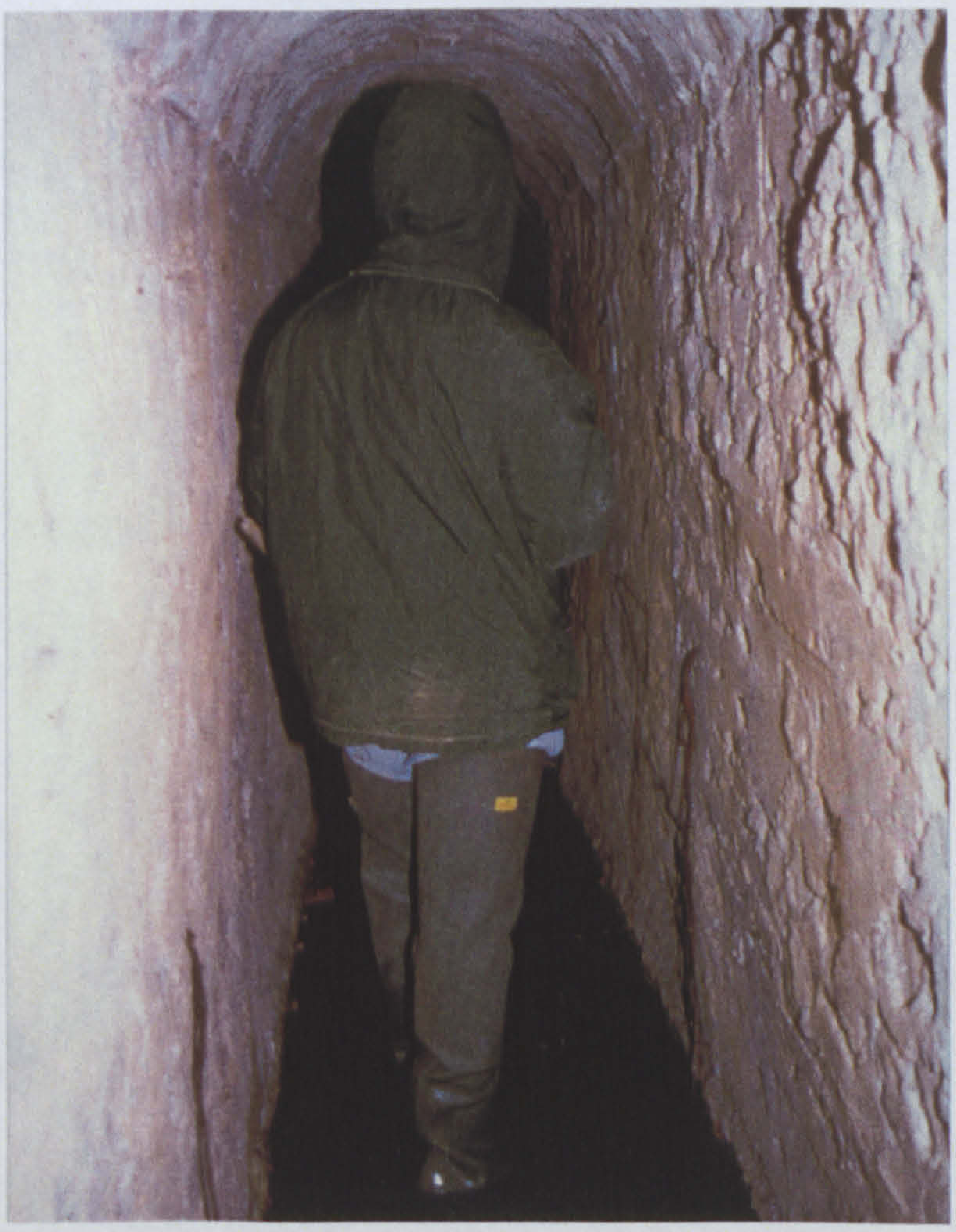

Plate 7.7. Above: the main roost entrance *(photograph by G. Mastrobuoni)*. Below: exploring the roost in January 1997 for a winter survey *(photograph by S. Viglietti))*.

Plate 7.8. The colony photographed in 1997. *Rhinolophus euryale* and *Miniopterus schreibersii* were associated in the same cluster *(photograph by S. Viglietti)*.

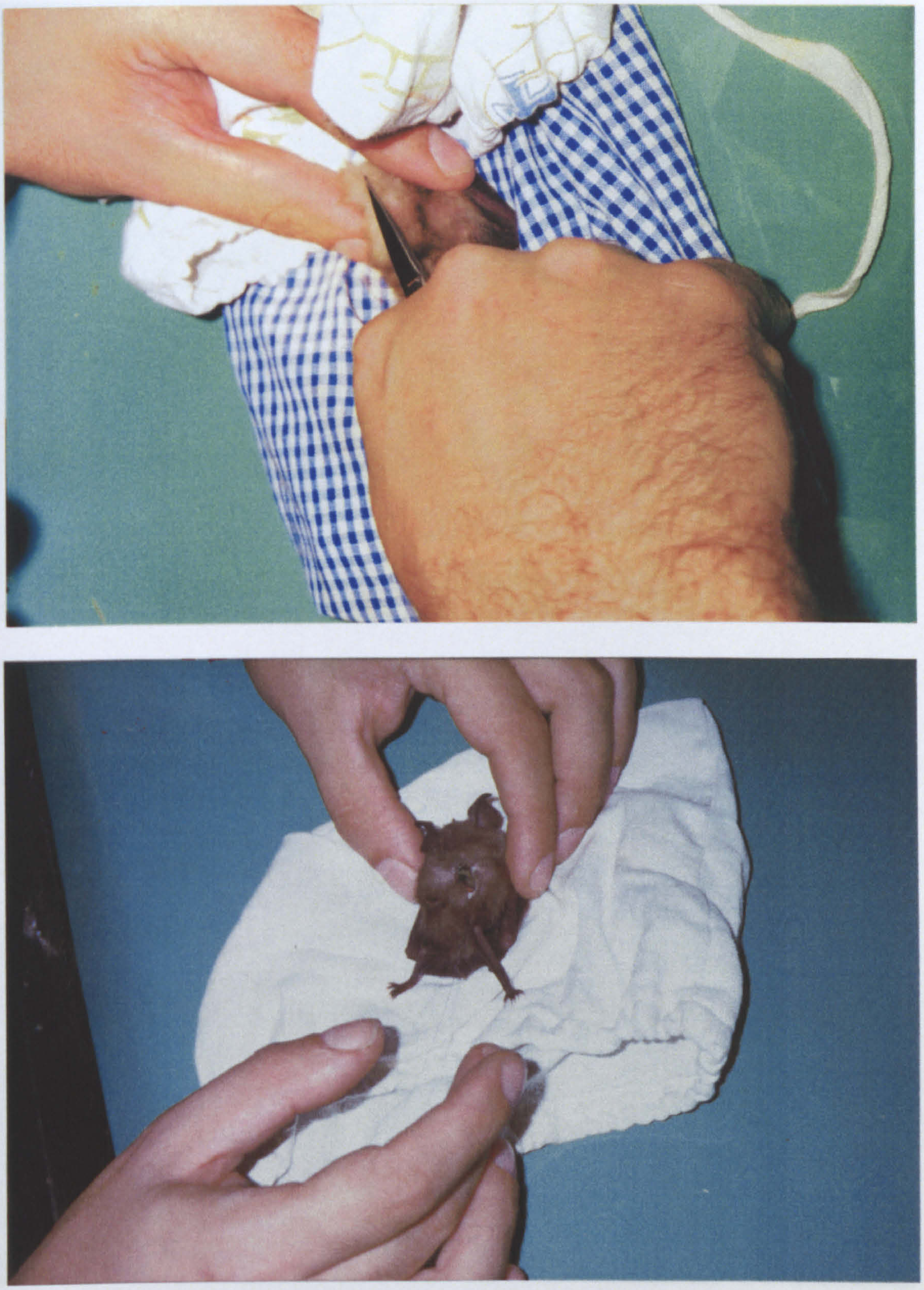

Plate 7.9. Tagging *Rhinolophus euryale*. Above: clipping the fur. Below: A tagged bat is ready to be released *(photographs by G. Mastrobuoni)*.

Plate 7.10. Above: a view of the broadleaved woodland area cleared in 1999-2000. Below: the bridge where *Rhinolophus euryale* roosted with other three species, located over 8 km from the main roost *(Photographs by G. Mastrobuoni)*.

Eight - Conclusions and recommendations for conservation of Italian bats

8.1. – Italian bats may be identified from their echolocation and social calls

In chapters 2-5 I described echolocation calls from 23 Italian bat species, and social calls from *Pipistrellus kuhlii*, *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. I also developed quantitative criteria for the identification of these species, showed that social calls from Italian pipistrelles are diagnostic, and that discriminant function analysis is a valuable tool to distinguish 18 species of bat by their echolocation calls. Some bat species may be unambiguously identified from spectral features of their calls, such as *Tadarida teniotis* and *Rhinolophus ferrumequinum*. At least in my study area, *Rhinolophus hipposideros* and *Rhinolophus euryale* may also be recognised according to the call frequency. Many other species emitted calls that overlapped in spectral and temporal parameters: hence, if qualitative, uncontrolled methods of identification are applied, there is a high risk of misclassification (chapter five). DFA permitted me to obtain good identification rates for most species emitting FM-CF calls, and for many of those producing FM pulses. The *Myotis* bats were the most difficult to tell apart, and for *Myotis mystacinus* and *Myotis blythii* identification rates were modest, although higher than those from random classification.

The resting frequency values obtained from *Rhinolophus mehelyi* and *R. euryale* showed some overlap, especially between juveniles of the former species and adults of the latter (chapter four). Overlap is also likely to occur between calls from flying bats, because the Doppler shift is likely to influence both species equally given the same habitat structure. It is unknown whether such overlap occurs where the two species occur sympatrically. Moreover, call frequencies from *R. mehelyi* are known to overlap those from *R. hipposideros* (Heller and Helversen, 1989). At least in Italy, however, sympatry of these three rhinolophids is unlikely to occur outside Sardinia (see chapter one). In fact, *R. mehelyi* is absent from northern Italy and is not sympatric with *R. euryale* and *R. hipposideros* in the south of the peninsula: the occurrence of *R. mehelyi* in Puglia, SE Italy, has not been confirmed recently (Marsico, 1998-99). In such areas there can be no risk of confusion in the identification of these species from their echolocation calls.

Because young rhinolophids emit lower frequencies than adults (Jones *et al.*, 1992; Jones and Ransome, 1993; Russo *et al.*, *in press* b; chapter four), *FMAXE* (frequency of maximum energy) is more variable during the late breeding season, when volant juveniles may be detected. Hence, at that time of the year, acoustic surveys are most likely to lead to wrong identification where rhinolophid species emitting similar calls are sympatric. As we have seen (chapter four), the clear *FMAXE* difference occurring between age classes in rhinolophid bats provides a means to distinguish juveniles from adults on roost emergence when acoustic techniques are coupled to emergence counts. A night-scope or video-camera should help correct counts for multiple passes. This method represents a completely non-invasive way to assess demographic variables such as population size and growth, and age class composition.

8.2. – Use of DFA: advantages and precautionary notes

Applications of multivariate discriminant functions in habitat use work are still not very popular among bat-workers. Indeed, researchers investigating habitat use and bat distribution on a community scale should consider devising their own functions. This approach is time-consuming, but worthwhile. According to the experience I acquired during this study and to work carried out by other researchers (Zingg, 1990; Vaughan *et al.*, 1997a; 1997 b; Parsons and Jones, 2000), some points may be summarised as a guideline for future developers of quantitative identification functions:

1) Neural networks generally provide higher identification rates (Parsons and Jones, 2000). DFA, however, is available from most statistical packages, and the underlying general concepts are well known to many researchers. Hence, DFA appears a 'more friendly' method and may result more attractive to researchers. DFA may also perform better than neural networks when small samples are concerned (S. Parsons, *pers. comm.*): this shortens the time required for obtaining sufficient samples and offers the possibility of covering rare species, for which a limited call sample is generally obtainable. However, large samples in discriminant functions should be obtained when possible because the more the calls are considered, the larger is the call variability covered (Vaughan *et al.*, 1997b). Quadratic discriminant functions provide more conservative results and are generally more robust statistically (Vaughan *et al.*, 1997b; this study).
2) Optimally, the discriminant function(s) to be applied in acoustic surveys should be developed from call databases from the same geographical areas (Vaughan *et al.*, 1997b; this study).

3) Call databases for the development of discriminant functions should include recordings obtained from bats leaving the roost when possible. Unlike recording on hand-release, obtaining calls from emerging bats does not disturb the animals and calls are often similar to search phase calls emitted in foraging situations. Even more valuable, from this perspective, are echolocation calls recorded in foraging grounds in association with species-specific social calls (see work on *Pipistrellus* spp. in this thesis, chapter five). Calls recorded from light tagged bats foraging in open areas may also be of use.
4) The bat detector response varies according to the brand adopted (Waters and Walsh, 1994; Parsons, 1996). Hence, calls recorded to develop identification functions should be obtained with the same detector to be used in subsequent identification work. An alternative is to obtain call samples including randomised recordings taken with detectors of various brands. Parsons and Jones (2000) developed neural networks and multivariate discriminant functions from a sample of recordings made with a Pettersson D980, with an Ultra Sound Advice bat detector S25 with PUSP, and even direct ultrasound recordings. This last option requires considerable extra effort.
5) In DFA (and in neural network applications, e.g. Parsons and Jones, 2000), a percent identification rate is obtained for each species. This offers an effective control over the data quality. Researchers may find it useful to fix the 'quality threshold' in practical applications, such as distributional or habitat use work. For instance, in chapter six, passes attributed to *M. blythii* were not considered for analysis at species level because the identification rate provided was judged unsatisfactory. Especially when investigating bat distribution (i.e. when even a single record may be important), a very conservative approach (i.e. rejecting identification when identification rates score <80 or 90%) may ensure a minimal risk of mistaken species recognition.

8.3. – Misuse of acoustic methods in identification of Italian bats

Although heterodyne has been largely used in Italy for bat identification (e.g. Violani and Zava, 1991), researchers should be aware of the important limitations of this technique (e.g. Vaughan *et al.*, 1997b). Time expansion detectors are costly (Jones *et al.*, 2000), but in the last years they have become increasingly popular among Italian bat workers. Despite this, the identification criteria applied in many studies published locally still remain unclear. The following example, chosen among many possible ones, will help clarify this matter. In a large-scale distributional work covering much of Italy presented to the first Italian Conference on bats (Castell'Azzara, March 1998), Fornasari *et al.* (1999) employed D940 and D980 Pettersson bat detectors in the heterodyne and time expansion modes. With reference to the acoustic identification procedures employed, in the 'Material and Methods' section of this paper it is stated (the original text is in Italian): '*Calls recorded in the field were identified by comparison with recordings made by Ahlén (1990) and Barataud (1996). In particular, for species emitting very similar calls such as* Plecotus auritus/austriacus *and* Myotis mystacinus/brandtii, *the Canary software was employed. Identification relied on parameters such as mean and variance of duration of calls and pauses between consecutive pulses recorded in heterodyne (Fornasari et al., in preparation). Features of spectrograms and power spectra from time expanded calls were also considered*'.

This statement raises several doubts in the reader's mind. Although the application of quantitative criteria is announced, these are not explained. The authors compare calls recorded in Italy with those of call libraries obtained elsewhere: the problems deriving from the different geographical origins of the unknown calls and those of reference are ignored. It is not clear why an analysis software was employed *only* to discriminate between the two pairs of cryptic species mentioned, and how these species were distinguished. To my best knowledge, the quantitative method the authors applied to heterodyne and presented as '*in prep.*' has not yet been published. In any case, heterodyne does not preserve the duration, frequencies and frequency-time course of the original signal (Parsons *et al.*, 2000), and hence the heterodyne output does not appear to be a good candidate for a quantitative identification method.

The misuse of ultrasonic detectors in studies conducted in Italy has induced a comprehensible diffidence in some researchers towards acoustic identification of bats (Lanza, 1999).

This is a pity, because acoustic identification is undoubtedly a powerful investigation tool as long as it is employed *scientifically*. It is a valid aid to identify a number of species with confidence (provided that it is used correctly and wisely), and may even lead to recognition of the existence of cryptic species, as in the case of *P. pygmaeus*, recently separated from *P. pipistrellus* (Jones and Parijs, 1993; Barratt *et al.*, 1997). On the basis of time-expanded calls only, in the present study it has been demonstrated that two such species occur in Italy (chapter three).

This thesis illustrates (chapters five) that DFA works well even when many bat species are included. Hence, DFA does appear a valuable means to investigate the speciose bat communities in the Mediterranean region with a rigorous approach.

8.4. – Habitat use by Italian bats: a community-scale perspective

In the study of habitat use, I pursued two different goals: to produce a broad picture of habitat preferences at the community level by means of acoustic surveys; and to concentrate, by applying radiotracking, on one particular species – *R. euryale* – because it is poorly known, rather uncommon in the study area and otherwise difficult to study. The results were discussed separately in chapters six and seven. It may be worth, now, summarising and discussing the information provided by the two investigations from the conservation perspective in order to identify conservation guidelines for Italian bats.

8.4.1. – Aquatic habitats and riparian vegetation

This study has demonstrated that aquatic habitats are important foraging habitats for Italian bats. Unfortunately, as was highlighted in chapter six, many Italian rivers are now subject to all sorts of mechanical and chemical degradation. Thus this important bat foraging habitat type is among the most threatened habitats occurring in Italy. My study also stressed that riparian vegetation is important, for three reasons at least:

1) Bats use riparian vegetation to forage. As discussed in chapter six, riparian vegetation supports a high density of prey insects. During transects, many bats were seen foraging near riparian vegetation. This was typical, for example, of *Pipistrellus spp. R. ferrumequinum* and *R. hipposideros*, species that were seldom encountered along transects, were observed foraging in aquatic habitats close to riparian vegetation (chapter six). Several *R. hipposideros* were repeatedly observed hunting at a specific foraging site by the *Salix* trees on the shores of the river Sangro, at the Abruzzo National Park (Russo, 2000) Riparian vegetation along the Sangro was also used by Natterer's (*Myotis nattereri*) and whiskered (*M. mystacinus*) bats (D. Russo, *pers. obs.*). Radio-tracked *R. euryale* exploited riparian woodland and scrubland to forage, in spite of the fact that the stream they visited was completely dry.
2) Riparian vegetation may protect foraging sites from the wind ensuring better conditions for hunting bats. In the two trawling species from the sub-genus *Leuconoe*, *Myotis capaccinii* and *Myotis daubentonii*, foraging activity

decreased as wind speed increased. As shown in chapter six, riparian vegetation may shelter the water surface from wind and protect suitable foraging sites.

3) Riparian vegetation is used as a commuting route. This study demonstrated that *R. euryale* follows riparian vegetation as a navigational landmark. It is probable that several other Mediterranean bats behave likewise, as in the case of Spanish *Miniopterus schreibersii* (Sierra-Cobo *et al.*, 2000).

8.4.2. – Woodlands

Woodlands constitute an important target for habitat management in relation to bat conservation because they provide many roosting and foraging opportunities (e.g. Mayle, 1990). Incorrect management of Italian woodlands has been addressed as a major threat to bats (Vergari and Dondini, 1998).

In the present study, broadleaved woodlands were used for foraging by most bat species, although only some of these showed a statistically significant preference. Pipistrelles often foraged in woodlands. *Castanea sativa* woodland managed for chestnut production, a typical southern Italy habitat, was found to be important for *P. pygmaeus* and, locally, for *Nyctalus leisleri* (but this observation may have been influenced by the presence of tree roosts, as discussed in chapter six). Beech woodlands of the Abruzzo National Park, among the best preserved of the whole country, are home to many bat species, including some of the rarest in Italy, such as *B. barbastellus*, *N. leisleri* and *M. nattereri* (D. Russo, *unpublished data*). In Tuscany, mature beech woodlands are frequented by the rare *Nyctalus lasiopterus* and *Myotis bechsteinii* (Vergari *et al.*, 1997; 1998). Data on rhinolophids obtained from acoustic surveys (chapter six) show that 25% of passes by *R. ferrumequinum* (n = 12) and 62% by *R. hipposideros* were recorded in broadleaved woodlands, and so was the only *R. euryale* pass detected. Radio-tracked *R. euryale* preferred above all broadleaved woodlands, which emerged as a priority foraging habitat for this species.

Transect work showed that conifer plantations, on the other hand, supported a limited number of species, and were used significantly less than other habitats by *H. savii*. Likewise, radio-tracked *R. euryale* never foraged in conifer plantations. As discussed in chapters six and seven, conifer plantations are not attractive to these bats probably because they support fewer insects; and *H. savii* might avoid such highly cluttered habitats, because its echolocation calls appear best suited to long-range detection in open areas. Conifers, except some scattered, rare endemic populations, are non-native and therefore undesirable trees in southern Italy.

8.4.3. – Arable land, olive groves

In southern Italy, farmland is often still managed traditionally, and as emphasised (chapters six, seven), this may account for the considerable use made of this habitat, at some of the study sites, by foraging bats.

Radiotracking showed that, in farmland, *R. euryale* avoided open fields, and concentrated foraging efforts close to tree lines and well-grown hedgerows (also used as commuting routes). Although no quantification was possible, qualitative visual observations conducted during transects suggested that other bat species also foraged preferentially close to such structures, as well as by isolated trees in open fields. Other favoured spots were those where manure had been recently added, as these probably attracted more insects. Acoustic surveys revealed a rather high bat activity in pure olive groves. Olive groves (in this case associated with small woodland patches and often adjoining large woodland areas) was significantly over-represented in *R. euryale* home-ranges, and ranked among the preferred habitats.

8.4.4. – Scrublands, grassland and bare areas

When planning acoustic surveys, I did not consider open sites such as grasslands and pastures because they were not dominant habitats in the study area and in fact I failed to identify six suitable sites which fitted the sampling protocol adopted (chapter six), i.e. sites that were homogeneous and large enough.

The only open natural habitat I considered, Mediterranean macchia (an interesting scrubland typology in biogeographical and conservation terms) was found to be of no special importance for bats. To my knowledge, before this study the occurrence of foraging *R. ferrumequinum* and *R. hipposideros* in Mediterranean scrublands had not been documented. Radio-tracked *R. euryale* avoided bare ground, scrubland and grassland sites with the exception of riparian scrublands. The latter mainly adjoined stretches of riparian woodland, determining a foraging and commuting habitat continuum for bats.

8.4.5. – Urban habitats

The present study dealt with rural towns rather than cities. Rural towns showed a high foraging activity from a relatively low number of species. Typical synanthropic taxa, such as *P. kuhlii*, *P. pipistrellus* and *H. savii* dominated foraging communities. As discussed in chapter six, the occurrence of vegetated areas and white street lights probably determined enhanced foraging conditions for such species, whose presence in this habitat is also made more likely by their tendency to roost in buildings.

Sensitive, less opportunistic species were not recorded in urban habitats. All radio-tracked *R. euryale* detoured to avoid settlements, and never foraged in urban sites. In conclusion, urban habitats may favour a few species but are probably harmful to most others.

8.5. – Guidelines for the management of bat foraging habitats in southern Italy and other Mediterranean areas

It is clear that several habitat types are important to foraging bats in southern Italy. This should be carefully considered in landscape planning and management. In conclusion, I will provide some guidelines emerging from my study for the management of feeding areas in southern Italy, and in similar areas elsewhere in the Mediterranean:

1) Aquatic habitats: these should be adequately protected. Pollution and structural alterations of rivers and lakes should be carefully avoided, and riparian vegetation should be preserved and, where necessary, enhanced.
2) Management of woodlands: timber harvesting should never create large clearings. However, the creation of some small clearings may increase bat activity and species diversity. Connectivity between woodland patches should be maintained. In any case, large, mature trees should never be removed because they may provide roosting opportunities. Reforestation should be conducted with native tree species, and conifers should be avoided. The worrying phenomenon of summer fires, which every year erases considerable fractions of the Italian woodlands, should be more effectively combated. Most Italian woodlands are subjected to the removal of old and dead trees to 'clean up' the sites and improve their timber productivity. The ecological importance of mature trees suggests that they should be left in place. Such trees offer precious roosting opportunities and sustain many insects. It is no coincidence that the area where

the tree-dwelling bat community is best preserved in central and southern Italy is the Abruzzo National Park, where mature and dead trees are left untouched. Bat boxes may increase roost availability, besides constituting valuable tools for distributional and ecological studies. Holes and crevices are typically scarce in conifer trees, and placing bat boxes in conifer plantations should offer a means to increase their attractiveness to bats. Cattle troughs in Apennine woodland habitats have proved crucial drinking sites for many bat species: these structures should therefore be kept in use, and where necessary further drinking facilities should be provided. The same goes for other habitats where water is scarce (e.g. Mediterranean macchia).

3) Management of farmland: traditional farmland should be encouraged. Farmers should be informed about the important role of bats in pest control, and bat boxes should be set up in agricultural land to provide roosting opportunities. The use of pesticides should be discouraged, while alternative pest-control methods and organic farming should be supported and disseminated. Olive groves may be important bat sites: in this habitat, where chemicals are commonly adopted against the olive flies, the use of pesticides should be reduced, and hedgerows and, where possible, some undergrowth should be kept.
4) Management of urban areas: because urbanisation is, in general, a threat to many sensitive bat species, the creation of new settlements either in the proximity of major roosts of endangered species or along main commuting roosts should be avoided. Encouraging stretches of vegetation in urban areas might provide more foraging opportunities and might even ensure connectivity along major navigation routes otherwise interrupted by the settlement. Special care should be taken in the management of urban roosts and in renovation of buildings currently or potentially suitable for bats.

Education campaigns could certainly play a major role in determining a greater public awareness of the precarious status of many bat species in Italy, and hence in pushing politicians to improve the legal background for bat conservation by including the protection of foraging habitats.

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