



The Norfolk Natterjack

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Norfolk & Norwich Naturalists' Society



... Researching
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Toad-in-the-hole...

Welcome to a New Year and hopefully one that will bring members together again if covid continues to become less of a problem. For the time being though Society talks and the AGM are still to be conducted on Zoom - please join in if you can. My thanks to all contributors to another interesting and varied 'Natterjack'. We have articles on growing a rare plant, further records of the rare Hoof Fungus gnat - maybe it will inspire you to find your own when walking around your local patch! Other articles invite members to become involved with the Broadland Country Park project or the exciting DNA barcoding analysis which can help naturalists ID species. Birds, bats, beetles and butterflies also feature. I would also like to draw your attention to the 'List of Recorders' where we welcome Liam Smith as the new Freshwater Fish recorder. Many of you will already be acquainted with Liam's underwater filming of local fish on the 'Norfolk Wildlife' Facebook Group so we eagerly await his reports. FF

Personal encounters with some of Norfolk's Wonderful 150...

Shepherd's Needle *Scandix pecten-veneris*. Robert Maidstone

My first encounter with this plant was a few plants in the edge of an open field in Wacton. They were almost in the grassy field margin and had been missed by any herbicides used on the field and were there for several years moving along the headlands, but in low numbers



Shepherd's Needle / Simon Harrap

Both Grace Corne and I had collected a few seeds when the plants occurred in our respective areas, Wacton and Sisland, and established a 'cultivated' colony. My colony is in a well manured and cultivated vegetable garden on clay soils; Grace has two distinct patches on light sandy soil, one colony is in gravel, the other in long established flower bed of low growing spreading plants.

The ability of the plants to grow in all these situation but being a rare and declining plant has puzzled me and I had made several observations over the last few years.

My colony and the one in the soil at Sisland have not expanded much and appear on the point of petering out. The ones in the gravel are flourishing

albeit with smaller plants, not so much goodness in gravel over a membrane.

Germination begins in late autumn with the winter rains and continues to February. The seeds appear to require shallow germination, they don't seem to need light but possibly a rich supply of air as the seed often tips down holes between clods or into wormholes. Germination is good in the colony in the gravel. This shallow need is reflected in my garden where deep buried seed don't seem make it, I don't get any germinated plants in the properly dug bits of the garden nor do seeds germinate the next autumn after the land is dug.

During a discussion with another NNNS member they mentioned the plant seemed to do very well in the cereal growing fields on heavy clay soils which seemed odd considering my observations in gravel. Back at home I consulted some of my farming text books and that seed bed requirements for autumn sown cereals "consists of fine material and lumps about fist size". In these clay soils cultivation is often shallow and done in autumn when the soil is still dry, the wheat and Shepherds Needle seeds drop between the clods onto the moist fine soil at the bottom and are protected by the bigger clods, the winter rains will break up the clods but not until later after the seeds have germinated.

With this knowledge I collected seeds from the gravel site in late October, some had started to germinate with a white root tip just visible, and I noticed most of the seeds stood upright between the stones and the root grew out from the top of the seed. Observation over the next few weeks shew the seedling grows from the top of the seed, arches over forming a loop that extends upwards drawing the seed leaves out of the seed husk. Seeds on their side encountered problems drawing the seed leaves out at right angles from the husk. Those seeds on the surface or held in loose soil often lifted the seed husk out of the soil where the husk dried and the seed leaves failed to emerge.

Thus one of the factors limited the spread of Shepherd's Needle's looks like the need of the seeds to be held upright in a moist but airy situation, gravels, clay clods and to some extent cracks and worm holes seem to facilitate seedling establishment. My well cultivated clay soil crumbles too well, closely covering the seed and excluding a high level of air.

I have several spare Shepherd's Needle seedlings which would be a good subject for experimental introduction to other places, like naturalist's gardens and gravel drives, to see how they survive.

I can be contacted at robertwmaidstone@gmail.com if anyone would like to take up the challenge.

Inspired by the account of Hoof Fungus *Fomes fomentarius* in Norfolk's Wonderful 150 and knowing that the Norfolk & Norwich Naturalists' Society is studying the new Broadland Country Park, when I found Hoof Fungus in the country park I emailed Tony Leech to check that it was known from there. I also mentioned that Sarah Burston, the park's manager, had told me about a rare fungus gnat recently found there. It was no surprise to learn from Tony that Hoof Fungus was already known from Broadland CP, and he also kindly sent me his text and Milly Kenward's photo of *Sciophila rufa* cocoons that later appeared in the November 2021 *Natterjack*, noted there as the second record for Norfolk and the third for England.

Armed with this pre-publication reference, I returned to Broadland Country Park on 23 September, leading a small group from Honeyguide Wildlife Holidays. We found Hoof Fungi on three standing stumps of birches *Betula* and on one of these, with about 30 'hooves' showing, we quickly found the distinctive fungus gnat cocoons shown in Milly's photo. It may be worth adding that there is both Silver Birch *B. pendula* and Downy Birch *B. pubescens* in the country park and the Hoof Fungi were all on Silver Birches.

On 5 November 2021, I was at Foxley Wood Norfolk Wildlife Trust nature reserve, again with a small Honeyguide group. I re-found a Silver Birch stump with *Fomes* that I remembered from a previous visit and, hey presto, there were identical cocoons. Could these be the third record of *S. rufa* for Norfolk and the fourth for England?

On 17 November, I returned to Broadland Country Park, this time with different Honeyguiders. We found more *S. rufa* cocoons and they were certainly on a different birch. Not only was it in a different place in the wood, this stump was long-fallen, not standing.



Sciophila rufa cocoons on Hoof Fungus, Foxley Wood, November 2021 / Chris Durdin

Finally, walking on Mousehold Heath, Norwich, on 28 November 2021 with my wife Julie, I returned to where I'd previously seen Hoof Fungi, close to Zaks



Hoof Fungus, close to Zaks, Mousehold Heath, on a fallen birch, with a single *S. rufa* cocoon. The fungus growth's change of direction shows that the stump fell off the main trunk several years ago. / Chris Durdin

Mousehold Diner. There were no cocoons by the fungi on the standing stump, though when I examined a fallen stump at the same spot there was a single cocoon under the overhang of a Hoof Fungus.

Given that *S. rufa* cocoons are distinctive once you've seen a photo, I wondered if they had been found several times in Norfolk once the *Natterjack* article had appeared. Not so, says Tony Irwin, at least from his inbox. I contacted national fungus gnat expert Peter Chandler, who confirmed the IDs, noting that: "There are similar species on other fungi but it's only likely to be *S. rufa* on *Fomes*."

From the above and Peter Chandler's information we have the following sequence of records for *S. rufa* in England.

1/ 23 September 2017, Flitwick Moor, Bedfordshire.

2/ 19 May 2019, between Stow Bedon and Thompson Common, Norfolk.

3/ 12 September 2021, Broadland Country Park, Norfolk.

4/ 5 November 2021, Foxley Wood, Norfolk.

5/ 17 November 2021, Broadland Country Park, Norfolk.

6/ 28 November 2021, Mousehold Heath, Norfolk.



Sciophila rufa 2020 distribution map, before these 2021 Norfolk records / Peter Chandler

My various photos show that the cocoons can be above, to the side of or below Hoof Fungi, sometimes touching, sometimes not, and on occasions a few centimetres from the fungus. They are usually by paler, younger *Fomes*, or at least *Fomes* showing recent growth, and are evidently comfortable close to the ground. It's also interesting to see how long the cocoons survive into the autumn. At Mousehold Heath, I had the impression that the cocoon had survived autumn rain and wind by being protected by the natural overhang of a Hoof Fungus.

I suggest that for anyone who has photos of Hoof Fungus, it could be worth checking them and more records of *S. rufa* may well come to light.

In any event, as Peter Chandler observes of *S. rufa*: "*It seems that it is now well established in Norfolk.*"

The next challenge is to return from May onwards and look for the larval webs, larvae or orange-coloured adult fungus gnats.

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Webb, Judy, A second English record of *Sciophila rufa*. *Dipterists Digest* 2019, Vol 26.

A few nice finds from Earlham Cemetery

Vanna Bartlett.

Earlham Cemetery, Norwich is my nearest green space and has served as my local patch for a good number of years. During the warmer months my recording efforts centre around solitary bees while the winter is a focus for hibernating ladybirds. Visually searching gravestones for these attractive beetles regularly results in some interesting new additions to the Cemetery invertebrate list of which these are a few of the highlights from 2021.

Lesser Thorn-tipped Longhorn Beetle *Pogonocherus hispidus*

One of the smaller Longhorn Beetles, this species is common and widespread but easily overlooked. They are well camouflaged at rest, resembling a bird dropping but are actually very attractive when examined closely. I first found them in the Cemetery in February 2021 with two on a gravestone under Beech trees. Searching the same area in November, I found two beetles on the 18th and six on the 19th, including a mating pair. Up to three were seen on various dates in December. It was interesting to find them on exactly the same gravestone as earlier in the year.



Lesser Thorn-tipped Longhorn Beetle tucked into an incised letter on a gravestone.



Lesser Thorn-tipped Longhorn Beetle showing off the long antennae that characterise this family (the Cerambycidae).



Ledra aurita final instar nymph, 4th December.

Images / *Vanna Bartlett*

Eared Leafhopper *Ledra aurita*

While searching gravestones for longhorn beetles, I was both surprised and delighted to spot the nymph of an Eared Leafhopper, *Ledra aurita*. This is one of our larger leafhoppers and although locally common across southern and central England it is seldom seen as it spends most of its time in trees where it is perfectly camouflaged on lichen covered branches, especially oaks. I have previously encountered adults on a handful of occasions when they have turned up in my moth trap.

Phloiophilus edwardsii

This little beetle is the sole species in its family (Phloiophilidae). They are only 2-3mm long and are infrequently encountered, with most records from the autumn/winter. They are associated with the dead, shaded out, fungoid lower branches of oak trees. There are a number of Norfolk records but this was the first from the Norwich area. Despite their small size they are quite distinctive but I was still lucky to spot one on a gravestone while walking through the Cemetery on Christmas Eve. Knowing what to look for, I then found a second individual a couple of days later.



Phloiophilus edwardsii



Epicaecilius pilipennis

Epicaecilius pilipennis

This attractive little Psocid or barkfly, as they are now commonly called, has a rather sparse and scattered distribution in the UK. Most barkflies are under-recorded and this one is no exception, being considered rather uncommon.

There appears to be only three previous Norfolk records for this species, the first in 2019 with two more in 2020, all from the Acle area, so it was very pleasing to add it to the Cemetery list with several individuals being found on grave-stones under Western Red Cedars on the 1st and 2nd of December. Many bark-flies are readily identifiable from the pattern of the wing venation and there is an excellent online key for the National Barkfly Recording Scheme (www.brc.ac.uk). *Epicaecilius pilipennis* has dark patterned wings and two light spots on the back of the head that distinguish it from other species.

My thanks go to Tony Irwin for confirming the identity of *Epicaecilius pilipennis* and to James Emerson for drawing my attention to the three previous Norfolk records of this species on iRecord. Thanks also to Martin Collier for information on *Phloiophilus edwardsii*.

Submerged Swallowtails

Kevin Radley & Hannah Breach

Through our studies of the Swallowtail butterfly *Papilio machaon* at Wheatfen (Ted Ellis Trust), Surlingham, we have found a number of their overwintering pupae over the last couple of years. Totally immobile and attached to the stems of fen flora (mostly, but not exclusively, Common Reed *Phragmites australis*), possibly for twelve months or more, those pupae spared the man-made 'predations' of various conservation regimes employed at reserves where the Swallowtail occurs (cattle/pony-grazing/trampling, mowing, brush-cutting etc.), are also at the mercy of more 'natural' faunal predators; and, furthermore, the effects of meteorological

conditions, often in conjunction with moon phase, on their environment.

Being directly connected to the tidal River Yare via various unregulated channels, Wheatfen is subject to regular flooding; particularly throughout the winter months. Completely submerged, this pupa (see photo), at 27cm above ground level, was photographed at high-tide near a December (2021) New-Moon. (Note caddisfly larva 'hitch-hiker' – inset). Although the pupae are able to endure a limited degree of submergence to no apparent detriment, this individual was, fortunately, exposed once again as water-levels receded at low-tide – until the next flooding *et cetera*.



A Swallowtail pupa that was submerged by the tidal river at Wheatfen with caddis fly larva attached (inset) / Kevin Radley

British Swallowtail now classed as vulnerable Mark Collins

Population data from the UK Butterfly Monitoring Scheme and other occurrence records have been used by staff at Butterfly Conservation to reassess British butterflies for a new UK Red List (Fox & Dennis in prep.). The last assessment, published in 2010, saw the British Swallowtail *Papilio machaon britannicus* classified as Near Threatened (Fox et al. 2010), but the recent analysis has raised the threat level to Vulnerable, based on an estimated one third decrease in abundance in the past ten years. This follows massive reductions in the occurrence of the species in 1 km² recording squares over the past half a century (summarised in Collins et al. 2020).

Now confined to perhaps 17 breeding sites in Norfolk, there is little good news for the Swallowtail. Its more robust continental cousin, *P.m. gorganus*, whilst not



A British Swallowtail - *Papilio machaon britannicus* / Mark Collins

yet challenging the Norfolk strongholds, is increasingly migrating to England's south coast, happy to feed and breed on a variety of common umbellifers while our own *P.m. britannicus*, depends entirely on Milk-parsley *Thysselinum palustre*. Milk-parsley is itself a Vulnerable species recently suffering a population crash in key conservation sites due to attack by an unidentified ascomycete fungus. The Fungus Specialist Group is

spearheading research to address the problem.

Meanwhile, the threat to the Swallowtail's breeding sites from sea-level rise and saltwater incursion, highlighted by Collins et al. (2019), has come into sharper focus with the conclusions of COP 26 and the detailed modelling by the Broads Authority's (2021) Broadland Futures Initiative.

A workable strategy for the Swallowtail's survival must surely involve not only continuing careful management in Norfolk's protected areas, but also translocation of both the butterfly and its foodplant to safe new sites further afield. The challenge is not helped by the memory of translocation failures at Wicken Fen in the 1970s and 1990s.

Year by year, we seem to be losing the very species that features in the Society's own iconic logo.

The 2021 Trustees Annual Report of the Norfolk & Norwich Naturalists' Society which had been included here in Natterjack156 as an 8-page insert (numbered separately) has since been externally examined and posted here on our website at

https://norfolknaturalists.org.uk/wp/wp-content/uploads/2022/09/NNNS_Annual_Report_2021.pdf

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Wasp Spiders

Irene Boston

The following photos show some of the (at least) 15 Wasp Spider nests, plus attendant females which were in the western car park area of the Norfolk Wildlife Trust's Roydon Common reserve earlier in the summer, 24th August and again 17th Sept. 2021.



The Wasp Spider is doing well in Norfolk since its first sighting at Saxlingham in 2006, as these images from Roydon Common show. / Irene Boston

I always enjoy a trip up to Cley, invariably there are birds there to photograph and enjoy. Daukes hide, the central one of three out in the middle of the marsh, is the place I aim for, it gives a good all round view, and the light is good, especially in the afternoons, anything could turn up here, some very rare birds have been seen over the years, but although its nice to see and photograph some thing unusual, its the more common species that predominate and that I enjoy photographing.

During late summer I had a very enjoyable few hours at this NWT reserve, ideally a bright day with a bit of hazy cloud is required, I was lucky, it stayed sunny with light cloud cover all the time I was there, and some beautiful birds turned up. Among the high lights was a party of four Greenshanks, one of my favourite waders, a most elegant bird, at one point all four were splashing and preening in shallow water, lovely to behold.



Greenshank in flight and female Kestrel with dragonfly prey
Marsh Harrier investigating possible prey item

A female Kestrel spent some time hunting dragonflies over the water just out front of the hide, then came in to perch on a fence post to eat them, that was a first for me, never seen that behaviour before. A Marsh Harrier flying down the edge of the open water spotted something floating and hovered over it before trying to pick it up, I couldn't make out what it was, probably a dead bird of sorts,

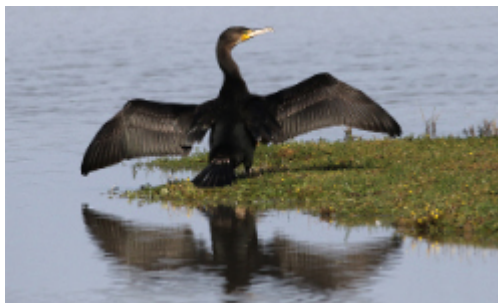


eventually the harrier lost interest and left it.

A Cormorant came in and landed on the island out front and proceeded to preen, holding its wings out in heraldic fashion from time to time, after a few minutes it proceeded on its way along the coast. Redshanks (cover picture) are one of our most common waders, subdued plumage, but with those bright legs unmistakable, there were several feeding out front mixed in with Black-tailed godwits.

So, - a very interesting experience, good weather, and some beautiful birds, always enjoyable.

Images / Tony Howes



Cormorant



A pair of Black-tailed Godwits

Black-headed Gull at 29

John Furse

A Black-headed Gull, ringed (ST 141436) as a youngster at Parainen, Finland on 12th June 1992 has once again returned to the same patch of grass in Sheringham (1st Nov 2021). The Helsinki Ringing Centre commented about the long-lived (29 years) and returning Black-headed Gull as follows:

"Excellent news, thank you! It is now #2 in Finnish longevity list (and #3 in Euring list)! Hopefully the patch of grass will feed it through the winter once again!"



The patch of grass where the gull was first seen in Dec. 2016



Images / John Furse

The Bat Conservation Trust operates the National Bat Care network with registered bat carers throughout the UK. Anyone who finds a bat can call the BCT Helpline and they will be given advice on how to contain the bat and the contact details of the nearest bat carer. In Norfolk, Abi Gray and myself are registered bat carers and we now have a small army of ambulance drivers to collect casualties. The majority of the casualties are Common and Soprano Pipstrelles and Brown Long-eared Bats, many of which have been caught and injured by cats, but every so often we open the box to find an unusual and unexpected species which requires careful identification.

This year was no exception. On April 3rd, I received a call from Stalham Veterinary Surgery saying that a bat had been brought in and needed help. The details passed to me on collection where that it had been found grounded on a patio in Stalham. I took a quick peep before taking it home, and thought that it might be a Serotine *Eptesicus serotinus*, although on the small side.

Back at home with calipers, digital balance, hand lens and keys for the identification of British and European bats to hand, I realised that it was a non-native species and keyed it out to Northern Bat *Eptesicus nilssonii*. Similar species are Serotine, Parti-coloured Bat and Savi's Pipistrelle, so to be absolutely sure, I sent off a faecal sample for confirmation by DNA analysis. Northern Bat has a wide European range from Scandinavia to Eastern Europe and south to eastern France and northern Italy. It is the only species that breeds north of the Arctic Circle. There is no evidence for fixed migration movements but isolated records from England, the Faroe Islands and from offshore installations in the North Sea indicate possible occasional migratory behaviour.



Northern Bat - a possible first for Norfolk - at least on the mainland /Jane Harris

This bat was a male and underweight at 7g (normal weight 9-13g), but uninjured, so I can only assume that it was exhausted and hungry after being blown off course or disorientated in some way during a foray over the southern North Sea. It was kept in care for several weeks while it gained weight and was tested for flying ability and stamina in the flight cage. It was released on the coast at Eccles on 28th May. There is much speculation about the influence of climate change on migratory bats. Perhaps bat carers will get more surprises when we open the box!



Mammals recorded at BCP

John Crouch

Mammal Species	Comment
<u>Rodentia:</u>	
Bank Vole (<i>Clethrionomys glareolus</i>)	common occurrence
Field Vole (<i>Microtus agrestis</i>)	common occurrence
Wood Mouse (<i>Apodemus sylvaticus</i>)	common occurrence
Yellow-necked Mouse (<i>Apodemus flavicollis</i>)	sporadic occurrence
Grey Squirrel (<i>Sciurus carolinensis</i>)	common occurrence
<u>Soricidae:</u>	
Common Shrew (<i>Sorex araneus</i>)	common occurrence
Pygmy Shrew (<i>Sorex minutus</i>)	common occurrence
<u>Mustelidae:</u>	
Stoat (<i>Mustela erminea</i>)	common occurrence
Weasel (<i>Mustela nivalis</i>)	common occurrence
Western Polecat (<i>Mustela putorius</i>)	sporadic occurrence
Eurasian Badger (<i>Meles meles</i>)	sporadic occurrence
<u>Cervidae:</u>	
Reeves Muntjac (<i>Muntiacus reevesi</i>)	sporadic occurrence
Roe Deer (<i>Capreolus capreolus</i>)	sporadic occurrence
Fallow Deer (<i>Dama dama</i>)	sporadic occurrence
Red Deer (<i>Cervus elaphus</i>)	sporadic occurrence
<u>Talpidae:</u>	
Common Mole (<i>Talpa europaea</i>)	common in parts of park
<u>Canidae:</u>	
Red Fox (<i>Vulpes vulpes</i>)	common occurrence
<u>Leporidae:</u>	
Brown Hare (<i>Lepus europaeus</i>)	one individual recorded
Rabbit (<i>Oryctolagus cuniculus</i>)	three individuals recorded
<u>Chiroptera:</u>	
Common Pipistrelle (<i>Pipistrellus pipistrellus</i>)	recorded in many areas of the park
Soprano Pipistrelle (<i>Pipistrellus pygmaeus</i>)	recorded in many areas of the park
Noctule (<i>Nyctalus noctula</i>)	recorded regularly high above the tree canopy
Serotine (<i>Eptesicus serotinus</i>)	recorded often in many areas of the park
Natterers Bat (<i>Myotis nattereri</i>)	recorded on two occasions near the entrance to the park
Brown Long-eared Bat (<i>Plecotus auritus</i>)	recorded often in the less dense wooded areas of the park

Table 1: List of Mammals recorded August - October 2021 at BCP

Summary:

There were not any Western Hedgehogs (*Erinaceus europaeus*) recorded which is surprising, but more research is being conducted and also into the occurrence of Chinese Water Deer (*Hydropotes inermis*) in the park as one is sure an individual of this species was recorded via night vision but the animal was in deep cover.

Bat recordings were taken during the period by employing an 'Echo Meter Touch' device fitted to an iPad.

Much more research needs to be done on the Bat species which frequent the Park particularly on the *Myotis* species; work will start in earnest when this order starts to emerge from hibernation early next year

There is a possibility that two other species may be located in the park, they are: Northern Water Vole (*Arvicola terrestris*) which occurs not too far away, the other being the Water Shrew (*Neomys fodiens*) which possibly already occurs.

The Harvest Mouse (*Micromys minutus*) has not been recorded on the list, though a nest was located by David Weaver.

Later in the year this species tends to build larger nests at ground level which are not dissimilar to that of the Field Vole (*Microtus agrestis*), so more research will be conducted next year by employing old tennis balls suspended in long grass/vegetation and bramble bushes.

Unfortunately the continued presence of uncontrolled dogs in the park is having a negative effect on the mammal species, particularly members of the Cervidae resident or visiting there.



New BCP Survey Transects now in place

Mark Collins (collinsmark@gmail.com)

An ambitious, wide-ranging programme of research is planned for 2022 in the Society's priority research site, Broadland Country Park near Horsford. First up is a *Breeding Bird Survey* in line with the established BTO methodology. An initial reconnaissance and two early morning visits for the dawn chorus in April and May will be led by Dave Weaver. An exciting bird list for 2021 is already in hand, including Woodlark and Nightjar breeding on the heath and winter sightings of Goshawk, Crossbills and Woodcock in the woodlands and wetlands.

Five 0.5km transects have been positioned in the Country Park, selected to maximise representation of the principal ecosystems and to offer a practical walking route. The transects begin by running south from the car park through the Sweet Chestnut Woodland, back along the Valley Mires, across to the Poplar and Birch Woodland and finally back northwards through the Conifer Woodland to pick up the Heath transect, finishing again at the car park.



Adding a nestbox along a new BCP transect / *Mel Collins*

Each section has been marked with 15 new nest boxes, all numbered, and GPS recorded. The fifth transect, across the Heath, will follow mown paths through the heather and gorse. A detailed guide map is now in preparation.

After the initial reconnaissance, a team of bird ringers led by UEA's Stephen Vickers will begin work with some mist-netting supported by temporary feeding stations in early Spring. Weekly checks for nest-building will be followed by ringing of fledglings throughout May. In June and July, the nest boxes will be emptied and cleaned, and sampled for insects and other invertebrates at the same time.

These transects will provide very useful routes for many naturalists with a half-day to devote to focused surveying and research in contrasting habitats. They are intended to complement the detailed 16-Compartment surveys that Specialist Groups and County Recorders are already conducting for mammals, insects and other invertebrates, vascular plants, mosses, fungi and many other taxa.

Don't forget that Andy Musgrove, Data Manager for the BCP project, is keen to receive and collate all your records. In due course they will be safely absorbed into the NBIS database. Special thanks go to Mike Riches for a magnificent campaign of nest box construction, Hans Watson and Dave Weaver for GPS and survey work and to all the team from BCP that have turned out to put the nest boxes in place.

What can DNA Barcoding do for you? *Janet Higgins & Yvonne Mynett*

Most of us will have heard of DNA barcoding or sequencing. In our own specialties we have all had species that have, frustratingly, undergone name changes, been split into two or more different species or have been lumped together. The cause of this is always cited as recent DNA evidence from an academic institution using methods seemingly out of the reach of us amateur naturalists. Traditionally the techniques used for DNA analysis have been far too expensive and complex for amateurs to consider but recently that situation has changed. Processes and equipment have been simplified to such an extent that DNA barcoding can now be done in a garage or kitchen. Consequently, NNNS and the Earlham Institute are developing a collaboration to enable NNNS members to gain an understanding how barcoding can be relevant to their interests as a naturalist. This is part of the Barcoding the Broads project (<https://www.earlham.ac.uk/barcoding-broads>) which has many aims from encouraging school children to explore their local biodiversity to working on specific projects with local naturalists alongside traditional methods of species identification. This

initiative is part of the public engagement component of the Wellcome-funded Darwin Tree of Life (DToL) project (<https://www.darwintreeoflife.org>).

Sam Rowe from the Earlham Institute has already run three workshops in the training laboratory on the Norwich Research Park, two focusing on plants and one focusing on insects. A total of 18 people have attended these workshops



Plant barcoding training session at the Norwich Research Park / *Tony Moyerley*

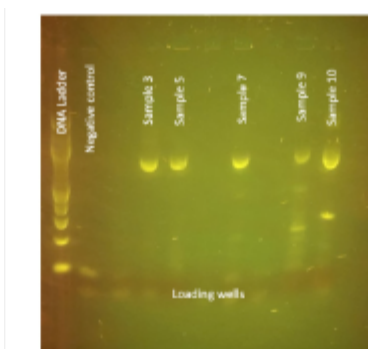
with a wide range of interests from plants, butterflies, fungi, badgers, insects and fresh-water ecology. They have come up with numerous ideas as to how barcoding can be used for natural history projects such as separating similar looking species, identifying different life stages of the same organism, identifying new species or contributing to the DToL project. The Norfolk Fungus Study Group (NFSG) have also obtained funding from the DToL public engagement scheme, with the help of RBG Kew and Sam, to develop a “barcoding in a box” package. This will include all the equipment needed for DNA barcoding but more importantly specific fungal barcode training sessions and online resources. The group will then be able to barcode their own specimens and contribute to the DToL project more effectively. Please email Sam at Sam.Rowe@earlham.ac.uk if you have any specific questions about DNA barcoding.

A typical barcoding training day

Training workshops run from 9:30am - 4:00pm with up to 9 attendees per session. The day starts with people either bringing their own samples or



Barcoding equipment
PCR machine (left) and Gel Electrophoresis tank/visualiser (right) / *Sam Rowe*



Gel from the Insect training session (17th November 2021)

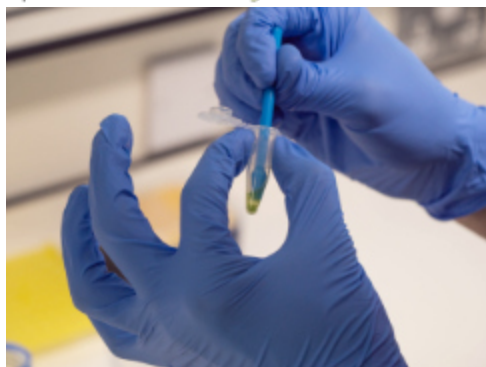
The DNA ladder contains DNA fragments of known sizes. Sample 3: *Hydrellia albilabris*, Sample 5: *Gyrinus substriatus*, Sample 7: *Nebria brevicollis*, Sample 9: *Baetis fuscatus*, Sample 10: *Tenuiphantes tenuis* / Sam Rowe

Norwich Research Park. We then return to the lab and Sam explains the steps involved while we get started by grinding up our sample to extract the DNA. The sample is then heated and centrifuged to remove the debris leaving the DNA in solution.

We then transfer a small aliquot of our DNA into a tube with the Polymerase Chain Reaction (PCR) mix and the primers for the barcode we have selected to use for our group of species. Just before lunch we load the PCR machine with all our samples. Sam then sets the program for the specific barcode we are using and we watch the first few temperature cycles as the barcode is copied, before enjoying our lunch together outside. When we return after lunch, hopefully there are millions of copies of the barcode from our sample. The next step is to load a small aliquot of our PCR product onto an agarose gel. Gel electrophoresis separates the DNA fragments by size, which enables us to see our amplified barcode as a bright green-yellow band on the gel. The gel takes around 30 mins to run so more time for explanation and discussion. Now with excitement and trepidation we go and



Sam captures the gel image with his mobile phone / Sasha Stanbridge



Extracting the DNA / Sasha Stanbridge

examine the gel, “has my sample worked?”. PCR can be a very fickle process and does not always work for many reasons. So far we have about 50% success with plants and 80% success with insects. This will improve the method is developed and the reason for failure can be traced.

Sam sends the samples which have a band on the gel off for DNA (Sanger) sequencing. He then emails us the sequencing files which are in a format ready for uploading to the DNA Subway: <https://dnasubway.cyverse.org/>. After uploading the files we follow the Blue Line, “Determine Sequence Relationships”. We can then compare the DNA sequence for our sample against online databases to find the most likely genus/species that our DNA came from. Some of the things we have successfully identified are: Spotted Medick (*Medicago arabica*), White Clover (*Trifolium repens*), Whirligig Beetle (*Gyrinus substriatus*) and Duckweed Leaf-miner (*Hydrellia albilabris*). We were also able to identify eggs scraped from a plastic pipe in a local lake as Pale Watery (*Baetis fuscatus*). The *rbcl* barcode was unable to distinguish Sea Campion (*Silene uniflora*) from other frequently occurring *Silene* species and we could not be certain which *Agrostis* species our grass sample was from.

Sam explains each step in detail giving plenty of background information, answering our many questions and encouraging us to make suggestions for future projects. The procedure has been developed to be simple and safe enough to be carried out by amateur naturalists at a local study site or work-room. If you are interested in getting involved or attending any future training sessions, please email Yvonne (stephen.pinnington777@btinternet.com) or Janet (jhiggins07@gmail.com).

Barcoding facts

DNA barcodes are universal but unique regions of DNA in the genome which have enough variability to identify most samples to the genus and species level. Regions of chloroplast genes, **rbcl** (RuBisCo - Ribulose-1,5-bisphosphate carboxylase oxygenase - large subunit) and **matK** (maturase K), are commonly used for barcoding plants. A region of the mitochondrial gene **COI** (cytochrome c oxidase subunit I) works well for barcoding animals. The nuclear **ITS** (internal transcribed spacer) barcode region is used for fungi.

PCR is used to create many (millions or billions) copies of the barcode region of DNA. The PCR products are then sequenced to determine the order of the **A**, **C**, **T** and **G** building blocks in DNA. Reference databases are used for the taxonomic identification of sequences obtained from barcoding. These databases contain the DNA barcodes assigned to previously identified taxa. The DNA barcode sequence is used to search the database for the most similar sequences, providing the most likely identification to the genus and species levels.

When barcoding is used to identify organisms from a sample containing DNA from more than one organism, the term **DNA metabarcoding** is used. This method is currently beyond the scope of Barcoding the Broads.



The next issue of 'The Norfolk Natterjack' will be **May 2022**

Please send
all articles / notes and photographic material
to the editor as soon as possible by
April 1st 2022 to the following address:

Francis Farrow, 'Heathlands', 6 Havelock Road, Sheringham,
Norfolk, NR26 8QD. Email: francis.farrow@btinternet.com

All photographs / images are very welcome, especially to accompany an article or document a record, occasionally however, because of space limitations, preference may have to be given to Norfolk-based images, or to those subjects depicting interesting or unusual behaviour, or are less commonly (or rarely) seen in print.

Membership subscriptions

The N&NNS membership year runs from 1st April to 31st March. During this time members will receive four copies of the quarterly newsletter, 'The Norfolk Natterjack', and annual copies of the Transactions of the Society, and the Norfolk Bird & Mammal Report. A full summer programme of excursions and a winter programme of talks are also organised annually.

New memberships and renewals can be made by credit card or 'PayPal' by visiting the Society's website at www.nnns.org.uk

Alternatively a cheque payable to
'Norfolk & Norwich Naturalist's Society' can be sent to:

Jim Froud, The Membership Secretary, Westward Ho, 4 Kingsley Road,
Norwich NR1 3RB

Current rates are £20 for individual, family and group memberships
(£30 for individuals living overseas).

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One of the mammals recorded at BCP - Fox (Drawing by Julie Curl)