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CONTENTS

TONI WATT – Changes in the zygopteran populations at Castle Fraser, Aberdeenshire with particular reference to *Coenagrion hastulatum* (Charpentier) (Northern Damselfly) 1

STEVE CHAM – Ovipositing behaviour, egg positioning and egg set size of *Chalcolestes viridis* (Vander Linden) (Willow Emerald Damselfly) at a new site in Britain 14

STEVE CHAM - Egg hatching, prolarvae and larval development time of *Chalcolestes viridis* (Vander Linden) (Willow Emerald Damselfly) in Britain 40

DAVID CLARKE - The last populations of *Leucorrhinia dubia* (Vander Linden) (White-faced Darter) at Claife Heights, Windermere, Cumbria? 60

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Cover illustration: Tandem pairs of *Chalcolestes viridis* on a branch of *Salix sp*. with oviposition scars. Photograph by Steve Cham.

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Changes in the zygopteran populations at Castle Fraser, Aberdeenshire with particular reference to *Coenagrion hastulatum* (Charpentier) (Northern Damselfly)

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Summary

The population of *Coenagrion hastulatum* (Northern Damselfly) has been surveyed at the Flight Pond at Castle Fraser for ten years but, since 2013, its numbers have declined. The other zygopterans present have shown a similar trend with the exception of *Enallagma cyathigerum* (Common Blue Damselfly), the population size of which has increased in recent years. These results are discussed in the light of changes in the vegetation around the Flight Pond and the arrival of *Coenagrion puella* (Azure Damselfly) in 2009. The creation of a second 'New Pond' has so far had a positive effect on the zygopteran populations at Castle Fraser.

Introduction

Coenagrion hastulatum (Northern Damselfly) is found mainly in northern Eurasia, where it can be abundant. At the southern edge of its range it reaches into eastern France and northern Italy but is also found in some localities at higher altitudes further south (Grand & Boudet, 2006; Dijkstra & Lewington, 2006). In the British Isles it is classified as 'Endangered' (Daguet *et al*., 2008; Taylor, 2008; Mill, *et al*., 2010) and is only found in north-east Scotland, where its distribution is scattered and locally abundant at some sites (Smallshire & Swash, 2014). Hickling *et al*., (2005) reported that it had expanded its range in the early part of this century in spite of having retracted northwards at its southern margin. However, over the next few years there were no noted changes in its range (Mill *et al*., 2010).

In Scotland, *C. hastulatum* breeds in sheltered pools and boggy lochans with abundant emergent vegetation and also in the shallow, sheltered margins of medium sized lochs in the Scottish Highlands. Females oviposit in tandem into both floating and emergent vegetation (Corbet & Brooks, 2008; Smallshire & Swash, 2014). including *Potamogeton polygonifolius* (Bog Pondweed) and *Equisetum fluviatilis* (Water Horsetail), the pair sometimes submerging. (Smallshire & Swash, 2014) The flight season starts in mid-May and extends until early August (Corbet & Brooks, 2008). In the boreal spruce forests of northern Sweden *C. hastulatum* is one of the most successful damselflies (Norling, 1984a).

In Scotland larvae live for two years (Smith & Smith, 1997) but cohort-splitting may occur because the larvae grow at different rates (Norling, 1984a,b, Johansson & Norling, 1994). In France the eggs hatch two or three weeks after being laid. There are 11 – 13 larval instars (including the prolarva) (Norling 1984a; Grand & Boudot, 2006). Grand & Boudot (2006) noted that the larvae live among submerged vegetation in shallow water. In Finland, in ponds where no surface water remains the larvae have been shown to be able to live for up to 12 weeks (Valtonen, 1986).

Coenagrion hastulatum is considered to be an ecologically sensitive species and hence may suffer as temperatures rise (Ott, 2005). Indeed, in Swedish populations, the length of the life cycle has been shown to increase with increase in latitude, with a one to two year life cycle in the south (58° 42’ N) and centre (63° 50’ N) of the country and three to four years in the north (67° 50’ N) (Norling 1984a; Johansson & Norling, 1994).

This study was carried out at Castle Fraser, a National Trust for Scotland (NTS) property in Deeside, Aberdeenshire. In October 2006, Bradan Aquasurveys surveyed three ponds for the National Trust for Scotland in Aberdeenshire, including the Flight Pond at Castle Fraser. The Flight pond was found to have high ecological value, leading to a further survey in the Spring of 2007 (Bradan Aquasurveys, 2007). Larvae of *C. hastulatum* were found in both years. These were the first records of this species at Castle Fraser. In 2008, adult *C. hastulatum* were seen flying around the pond (J. Dinning, pers. comm.). Since 2009, detailed surveying has been carried out by Dinning and then by the NTS Rangers (Dinning, J., 2009-2016). A report was provided for 2012 by Blyth (2014)

Site

Castle Fraser was given to the National Trust for Scotland (NTS) in 1976 with 10.52 hectares of surrounding policies (grounds and gardens). The NTS purchased an additional 131ha in 1993, which included the Flight Pond.

Flight Pond

The Flight Pond (NJ724134) was created in the 19th century in an area of low-lying bog to attract wildfowl for shooting. Although originally round, today the pond is long and thin, approximately 260 x 26 metres, covering an area of about 0.5 hectares, with a silt substrate. The maximum water depth is 1.9m with an average depth of about 1m. The small amount of sediment in the pond has a maximum depth of 50cm and a mean depth of 21cm (Luxmoore, 2008).

The most abundant emergent plants are *Carex rostrata* (Bottle Sedge), *Eleocharis palustris* (Common Spike-rush) and *Equisetum fluviatile* (Water Horsetail). *Carex rostrata* grows along the south-west margin of the pond; also in the east end of the pond where there is a large bed of it growing next to an area of *Eleocharis palustris*. *Equisetum fluviatile* is mainly confined to several beds in the centre of the pond. Much of the deeper parts of the pond are covered in *Potamogeton natans* (Broad-leaved Pondweed) (Waterside Ecology & Aquaterra Ecology, 2014).

On the south side of the pond there is an extensive area of marsh with no obvious access through it, although a short floating walkway has been made to aid recording and access. The north side of the pond is very accessible, with a sloping bank to the pond and a waymarked path leading along the length of it, forming part of the Alton Brae Trail. The north side of the pond was surrounded by dense *Picea abies* (Norway spruce) with pockets of *Picea sitchensis* (Sitka spruce), which were planted after the 1953 storms blew the existing trees down. However, half of the Spruce plantation has recently been felled (October 2019.)

New Pond

Concerns had been raised over the *Coenagrion hastulatum* population in the Flight Pond from about 2006. This was primarily due to the presence of goldfish in the Flight Pond which, at one time, were present in quite large numbers (Blyth, 2014). Since removing the fish would be too difficult, it was decided to create another pond within flying distance of the Flight Pond. The site chosen was determined by existing constraints, including archaeology, rig (ridge) and furrow farming systems, and badger setts.

Funding was obtained and the New Pond was dug on 12 March 2011, about 80m from the Flight Pond and hidden in a low dip in the land. An area was chosen where there was a ditch inflow to the pond and an outflow, as this seems to be a recurring factor in ponds containing *Coenagrion hastulatum*. The pond is 30m x 15m (Blyth, 2014) with a maximum depth of 1.5m and with gradually sloping sides. Vegetation was left to colonise naturally as a biosecurity measure to avoid contamination with goldfish eggs from the Flight Pond.

Methods

Flight Pond (Plate 1)

Since 2009, detailed surveys have been carried out on the adults of all the zygopterans present, using the guidelines of the Dragonfly Monitoring Scheme 2009 Pilot (BDS, 2010). Dinning set up two 50m transects and two 100m transects along the northern edge of the Flight Pond. Fortnightly surveys were carried out from May until September each year from 2009. In 2017 the surveys were taken over by the NTS Ranger Service. The maximum number of adults of each species of zygopteran counted during any one transect was recorded.

New Pond (Plate 2)

Initial surveys were carried out on both larvae and adults at the New Pond in 2012 by Blyth and Watt (Blyth, 2014). However, it was only in 2018 that this pond was included in the dragonfly recording programme, when a transect was set up around the whole pond, using the same method as used for the Flight Pond.

Fixed point Photography

Fixed point photography was set up at three sites around the Flight Pond in 2002 to record long-term changes in the vegetation around the pond, and takes place every five years. The New Pond was incorporated in 2017, using four sites around the pond margins.

Results

There are 10 species of adult odonate recorded at both the Flight Pond and the New Pond - six zygopterans (including *Coenagrion hastulatum* (Plate 1)) and four anisopterans. The other zygopterans are *Enallagma cyathigerum* (Common Blue Damselfly), *Pyrrhosoma nymphula* (Large Red Damselfly), *Ischnura elegans* (Blue-tailed Damselfly), *Coenagrion puella* (Azure Damselfly) and *Lestes sponsa* (Emerald Damselfly). The four species of anisopteran are *Libellula quadrimaculata* (Four-spotted Chaser), *Aeshna juncea* (Common Hawker), *Sympetrum striolatum* (Common Darter) and *Sympetrum danae* (Black Darter).

Plate 1. The Flight Pond in August 2019.

Plate 2. The New Pond in August 2017.

A

B

Plate 3. (A, B) Males of *Coenagrion hastulatum*.

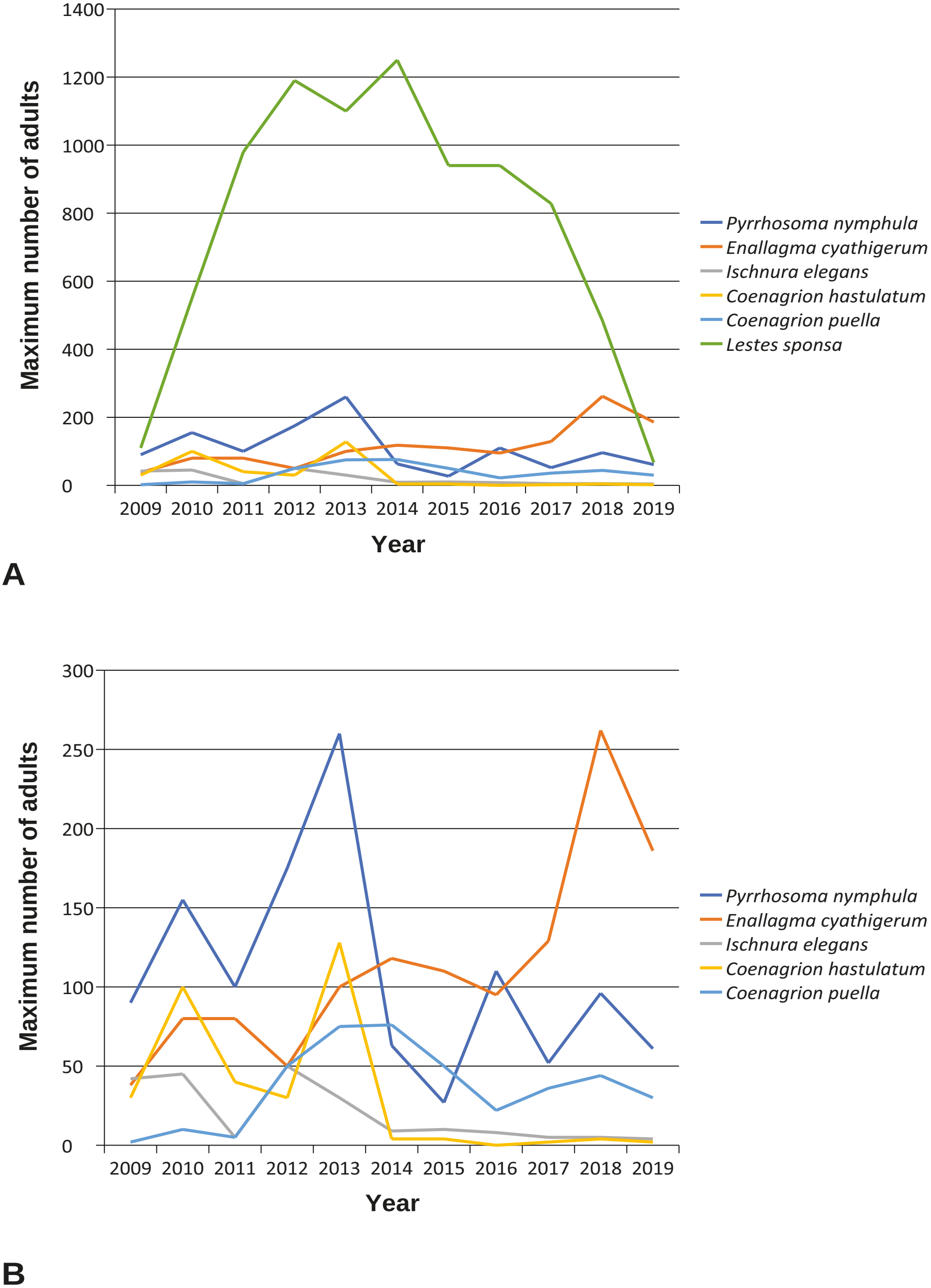

Figure 1. The maximum number of flying adult zygopterans recorded in any one transect at the Flight Pond. A) All six species; B) With the data for *Lestes sponsa* removed.

Table 1. The maximum number of flying adult *Coenagrion hastulatum* counted in any one transect at the Flight Pond.

2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
30	100	40	30	128	4	4	0	2	4	2

Flight Pond

From the maximum number of adult *Coenagrion hastulatum* on the wing counted along any one transect, it can be seen that the population size has been very variable. Prior to 2014, the numbers were large with a maximum count of 128 (in 2013), whereas after this the numbers declined dramatically, with no more than four being counted in any given year from 2014 to 2019 (Table 1, Fig. 1).

In the other zygopteran species, there was a decrease in population size after 2013/2014 in all except one, i.e. *Enallagma cyathigerum*, which showed a marked increase, especially between 2016 and 2018 (Fig. 1). The greatest proportionate decrease occurred in *Lestes sponsa*, which increased in numbers between 2009 and 2014, reaching a peak of over 1,200 that year before dramatically declining to less than 100 by 2019. *Pyrrhosoma nymphula* showed a decrease after 2013 but there is evidence of some recovery from 2016 onwards. *Coenagrion puella* showed a steady increase from 2009 to 2014, then decreased in 2015 and 2016 before showing a slight recovery in the following years. *Ischnura elegans* followed the same trend as *C. hastulum* after 2014, with very few numbers recorded since then (Fig. 1).

Fixed point Photography The photographs show dramatic changes to the vegetation on the banks. In 2002 there was virtually no birch regeneration on any of the banks. However, by 2017, when the last fixed-point photographs were taken, there was birch regeneration on all sides of the pond, some plants having become large trees, approximately 8-10 meters tall. On the south facing bank the birch is causing shading of the pond (Plate 4).

Forestry Work Since the birch on the south-facing bank mentioned above is causing shading it is gradually being thinned. In October 2019, at the eastern end of the north bank, half the spruce wood was clear-felled, partly because the trees were starting to die and fall over towards the footpath and the pond; also, the trees were on old rig and furrow, mediaeval farming systems which the NTS archaeologists did not want to be damaged by the roots of falling trees.

New Pond

In 2012 the most abundant adults were *Lestes sponsa* and *Pyrrhosoma nymphula*, with maximum counts of 100+ and 30+ respectively (Blyth, 2014).

Larvae of *Coenagrion hastulatum* were first recorded in 2013. In 2015, exuviae of all 10 species of odonate were found in a survey, confirming that all were breeding in the New Pond.

It is too early to determine any trends in zygopteran populations in the New Pond. However, the numbers of *Coenagrion hastulatum* are promising, with a maximum count of 29 adults seen in any one survey in 2018 and 10 adults in 2019.

Discussion

Although it is possible that the reduction in numbers of *Coenagrion hastulatum* may be a result of climate change, with the average temperature in Scotland increasing, it seems unlikely to be the only factor since other species with a wide geographical range, such as *Ischnura elegans*, are also decreasing in the Flight Pond. Furthermore, the nearby New Pond appears to be supporting a reasonable number of *C. hastulatum*. It is possible that changes in the vegetation and/or the increase in shading are having a negative effect and this could be due to the direct physical impact of shading of both the Spruce and the Birch, or to other changes in the physical environment, such as increased leaf litter in the water or the drying out of the marsh area. These factors could also be the reason for the decline in the population sizes of *C. hastulatum*, *Ischnura elegans* and *Lestes sponsa*.

Coenagrion puella is increasing its range and it was first recorded at the Flight Pond in 2009. It was then a rarity in northeast Scotland and it is thought that Castle Fraser was the most northerly population recorded at that time. In 2013 it colonised a pond at Crathes Castle, 23.5km south of Castle Fraser. There are still few records in northeast Scotland and only about five records shown on the NBN atlas in the far north of Scotland along the coast from Inverness and northwards. The increase in its population size at the Flight Pond may be having a negative impact on the *C. hastulatum* population.

In Sweden there appears to be no correlation between the abundance of fish and of *C. hastulatum* larvae (Johansson & Brodin, 2003), whereas *Aeshna juncea*, which is present in both ponds at Castle Fraser, has been shown to prey on *C. hastulatum* larvae (Johansson, 1993).

There is concern that *C. hastulatum* could be lost from the site, particularly as the numbers of adults recorded at the Flight Pond have been so low since 2014. However, the New Pond has been a success and the creation of further ponds may improve the site further. The previously-known Deeside populations

A

B

C

D

Plate 4. Fixed point photographs of the Flight Pond. (A) 2002, (B) 2007, (C) 2012, (D) 2017. Note the increase in birch regeneration, particularly between 2002 and 2007.

of *C. hastulatum* are centred on Dinnet, which is about 30km southwest from Castle Fraser. Hence, if the population at Castle Fraser is lost, it is unlikely that re-colonisation will take place.

The increase in the population of *Enallagma cyathigerum* is interesting and it is possible that it is out-competing the other zygopteran species. In any event, it would be helpful to know why this species is so successful at this site.

The New Pond has clearly had a positive impact on the odonate fauna at Castle Fraser since all 10 species have been confirmed as breeding there. Long term, for both *C. puella* and *E. cyathigerum*, it will be interesting to monitor changes in their populations in the New Pond with particular respect to *C. hastulatum.*

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Ovipositing behaviour, egg positioning and egg set size of *Chalcolestes viridis* (Vander Linden) (Willow Emerald Damselfly) at a new site in Britain.

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Summary

Females of *Chalcolestes viridis* (Vander Linden, 1825) (Willow Emerald Damselfly) oviposit into the branches of woody plant species above water or wet ground, a behaviour that is different to any other British odonate species with endophytic oviposition. At some new sites, populations of *C. viridis* can quickly build up to reach high densities. During 2020 high numbers of tandem pairs were observed at a site in Bedfordshire, England, where the availability of suitable oviposition substrates was at a premium, resulting in branches being covered by oviposition scars. These oviposition scars are in lines and each one is the result of a single oviposition event in which a set of eggs is laid. The terminology is discussed. The structure of the ovipositor and the process of oviposition are described.

Introduction

Chalcolestes viridis (Willow Emerald Damselfly) is a common and widespread species across continental Europe. Since its first appearance in the UK in 2007 it has spread across southeast England and is now further expanding its range west and northwards (Parr, 2020). This range expansion has enabled the colonisation of a new site to be studied. Following the first records at Stanford Lake, Bedfordshire in 2019 it was confirmed as a breeding species in July 2020, when emergence and exuviae were recorded (Cham, 2021a). Throughout August a sizeable population was present at this site, with numerous tandem pairs (recorded as E (101-500) using the BDS convention) ovipositing into the branches of *Salix caprea* (Sallow) and *Salix* spp (White or Crack Willow), hereafter referred to collectively as *Salix*. During the same period, *C. viridis* was also recorded at other sites across Bedfordshire, but in much smaller numbers, and it remains to be seen if the results from this study site are typical of other populations.

As with other lestid species, *C. viridis* remains in tandem after copulation, and oviposition follows soon after, with egg sets deposited along a distinctive line (Matushkina & Gorb, 2000). Females may oviposit in tandem or alone. In both cases they adopt a characteristic pose and the degree to which they bend their abdomens is considered to be related to the stiffness and force required to penetrate the substrate (Matushkina & Gorb, 2007). This differs between lestid species and reflects the type of substrate used for oviposition. Egg sets of up to eight eggs, laid in a characteristic pattern (two pairs of four), have been reported in Europe (Matushkina & Gorb, 2000), with oviposition rates for a number of Zygoptera species showing that lestid species oviposit more slowly than others, *C. viridis* being the slowest of those studied, with a rate of 1.0 egg/minute (Table 3 in Matushkina & Gorb, 2000). The structure of the ovipositor has important features enabling *C. viridis* to oviposit into stiff woody substrates. With the high density of oviposition scars on the new growth of *Salix* bushes at Stanford Lake, the positioning of eggs and the egg set size was investigated and compared with previous studies in continental Europe.

Methods

The study site was at Stanford Lake (TL159407) and visits to observe *Chalcolestes viridis* were undertaken at intervals from June through to September 2020 and made as frequently as possible from when the first newly emerged teneral adults were first seen during July through the period of peak activity in early August and into September. Visits were made at various times during the day to assess diel activity patterns: 8.30-12.30, 13.00-17.00 and 19.00-20.00 (BST). All parts of the lake receive full sun at some time during the day in the summer months and each was inspected for activity. Oviposition behaviour in the field was recorded by still photography and by video using a Canon M6 mkII camera and 100-400 mm lens. Videos of ovipositing behaviour were taken at 4K at 25 frames per second, allowing a maximum recording time of 29 minutes and 59 seconds. Oviposition behaviour and egg set size were later reviewed using the video timeline to note the time taken to deposit a set of eggs and the interval between them. Close up images of the oviposition process and the female ovipositor were taken with either a Canon EF100 mm L Macro IS or MPE 65 mm 1-5x lens to compare with morphological studies of other lestid species (Matushkina & Lambret, 2011).

Small samples of fresh growth *Salix* (Willow) branches of varying diameters with oviposition scars were collected for evaluation of egg density. A novel method of illumination was developed to show the insertion point formed by oviposition and to correlate it with the bark's surface topography and egg set location. This technique required removing a small sample of *Salix* bark with oviposition scars, cutting around the circumference of the bark at either end between leaf buds and then making one longitudinal cut between them. The bark could then be cleanly stripped away from the heartwood in one piece. Each bark sample was placed on a standard glass microscope slide and held flat by sticky tape at either end. The glass slides holding the samples were photographed by switching between two illumination methods: i) two LED lamps (Ikea Jansjo) positioned behind the sample to provide strongly directional backlighting from either side and ii) frontal lighting using two LED video lights (Neewer 480 light panel) to illuminate the bark surface. With this simple method the backlighting revealed the egg sets beneath the bark surface, which could then be exactly correlated with the bark surface topography shown by the top lighting.

To measure egg density, a piece of black card with a 1cm x 1cm square aperture was placed onto each bark sample to act as a mask and backlit to enable counts of eggs within the aperture. The egg count within the aperture represented the number of eggs/cm^2. Measurements of the distance between egg insertion points were also recorded. Several of the branches with oviposition scars were dissected to assess the positioning of eggs in the outer bark layers.

As the common British name suggests, *C. viridis* has a strong association with *Salix* spp, although an increasing number of other plants are utilised for oviposition (d'Aguilar *et al*., 1986; Parr, 2016). The discovery of slight blister-like mounds on the stems of *Lythrum salicaria* (Purple Loosestrife) represented a new, unreported plant species utilised by *C. viridis*, enabling comparison with *Salix* as an oviposition substrate. Samples of stems of *L. salicaria* were taken but the outer layers proved less easy to remove intact, thus requiring further dissection to reveal the egg sets.

Additional visits to Stanford Lake were made during late September and early October 2020 to assess the development of oviposition scars and *Salix* growth after the adult flight period had come to an end.

Terminology

In recent publications both 'gall' and 'scar' have been used for the raised area resulting from oviposition at each insertion site. 'Egg galls' were mentioned by Kirby (1908) and Tillyard (1917) and 'galls' is used in d'Aguilar *et al.* (1986), Askew (1988), Brooks, *et al.* (2014) and Smallshire & Swash (2018), while Grande & Boudot (2006) state that oviposition leaves blistered marks clearly visible in the bark (galles). 'Scars' is used by Smallshire & Swash (2020) and 'track-like scars' is used by Dijkstra & Lewington (2006) and Dijkstra, *et al*. (2020). Parr (2016) uses 'oviposition scars' while Tyrrell (2019) refers to both 'oviposition scars' and 'egg scars'. The series of oviposition marks have been referred to as ‘clutch lines’ (Matshukina & Gorb, 2002; Matushkina & Lambret, 2011), ‘chains’ (Matushkina & Gorb, 2000), egg tracts (Parr, 2016) and ‘oviposition scar tracts’ (Tyrrell, 2019).

This terminology is confusing. Hence in the light of the observations of oviposition made during this study and in an attempt to achieve conformity, the following definitions are suggested:

- Oviposition scar – the raised mound formed where an incision has been made by a female to lay eggs in woody material (Plate 1(i)).
- Egg set – The eggs laid by a female in a single incision (Plate 1(ii)).
- Oviposition line – the linear row of oviposition scars made by a female laying a batch of egg sets (Plate 1(iii)).

Plate 1 The outer bark layer of a branch of *Salix* sp. showing (A) the surface topography and incisions (insertion points) with associated scarring and (B) the corresponding underlying egg patterns. (i), two incisions with raised oviposition scars, as seen from the surface of the bark, (ii) one egg set inserted in a single incision and comprising two eggs on either side of the insertion point, i.e. a 2+2 pattern, (iii) an oviposition line resulting from a number of incisions made on a single visit by a female.

Results

Observations

Stanford Lake is a shallow lake subject to significant fluctuations in water level. As a result, two marginal rows of *Salix* have developed, one of mature trees representing the former lake margin when water levels were high and the other the current lake margin at lower water levels. Older oviposition scars from 2019 could still be seen on overhanging branches of the older tree branches. These trees were also utilised by adults to roost high up. Following some clearance work over the 2019/20 winter period, new growth of *Salix* was much in evidence during 2020. This was very attractive to *Chalcolestes viridis*, with tandem pairs ovipositing frequently into the branches. The current southern lake margin and, to a lesser extent other margins, have extensive growth of *Lythrum salicaria* which was also frequently being utilised by *C. viridis* for resting and oviposition.

From 5-12 August 2020 very warm weather conditions were experienced with afternoon temperatures exceeding 30°C daily. On 13 August the weather changed, with heavy and torrential downpours which curtailed observations, and this was to be the weather pattern for several days. Thereafter, daily weather was variable but allowed visits to continue during sunny periods. Daily visits revealed peak activity of *C .viridis* to be in the afternoon.

The northern margin of the lake was in full sun for most of the day and has new *Salix* growth along much of its length. As noted above, the southern margin has extensive growth of *L. salicaria*. It is interspersed with *Salix* and was in full sun from 10.30 until the evening. Visits in the mornings to any part of the site often failed to record more than a few single males and an occasional female, despite other odonate species being much in evidence. During afternoon activity many tandem pairs (D (21-100) - E (101-500)) were abundant in the areas of *Salix* spp. and *L. salicaria*. Tandem pairs were frequently observed arriving to oviposit, even in new growth branches already covered with recent and extensive oviposition scars (Plate 2). Evening visits recorded far fewer tandem pairs but a greater frequency of females ovipositing alone. Single females were rarely observed during the day but frequently in tandem with males.

On the evening of 10 August it was especially warm and humid. A visit at 19.00 recorded single females (counts in the C (6-20) range) ovipositing into *Salix* branches uninterrupted by the attention of males. A few single males were seen high up in Willow trees further up the bank. On 11 August many tandem pairs were egg laying in the afternoon, although a visit that morning had recorded none. On 12 August between 12.00-13.00 only two single males were seen during a visit in the morning with many tandem pairs recorded during the

Plate 2. Tandem pairs of *Chalcolestes viridis* were frequently observed ovipositing into branches of *Salix* sp. (Willow) which were already covered with oviposition scars.

afternoon visit.

Single ‘floating’ males were often seen during the afternoon, presumably searching for unpaired females. At high population density the competition for females was high, leading to mistaken mixed pairings. On one occasion, a male was seen attempting tandem with a male *Lestes sponsa* (Emerald Damselfly) and on another an attempted copulation with a female *Erythromma viridulum* (Small Red-eyed Damselfly) after it had taken it in tandem (Plate 3). Furthermore, on several occasions single males were observed attempting tandem with the male of an existing tandem pair.

Ovipositing tandems were attracted to new growth of *Salix*, and numerous pairs could be seen and heard within various bushes. Outdoor temperatures over the initial part of August exceeded 30°C, with activity at its greatest in the centre of bushes where it was cooler and possibly preferential for egg survival. The branches in the centre of *Salix* bushes are of larger diameter than more peripheral ones. The movement and flight of the many tandem pairs searching for suitable branches could be heard as their wings came into contact with

Plate 3. At times of peak activity mistaken mixed pairings were observed. Here a male in an unusual mixed tandem with a female *Erythromma viridulum*.

branches and leaves. Most of the branches were covered with oviposition scars and numerous oviposition lines. There was a strong preference for branches that were either overhanging water or in very close proximity to wet ground. Branches on the same bush but further away from water had few or no oviposition scars (Plate 4).

On days of inclement weather, tandem pairs and individuals were recorded during brief sunny intervals on the lower *Salix* bushes by the lake. As the weather changed to rain or cloudy conditions they were seen flying up into the taller trees where they presumably also roosted overnight.

Structure of the ovipositor

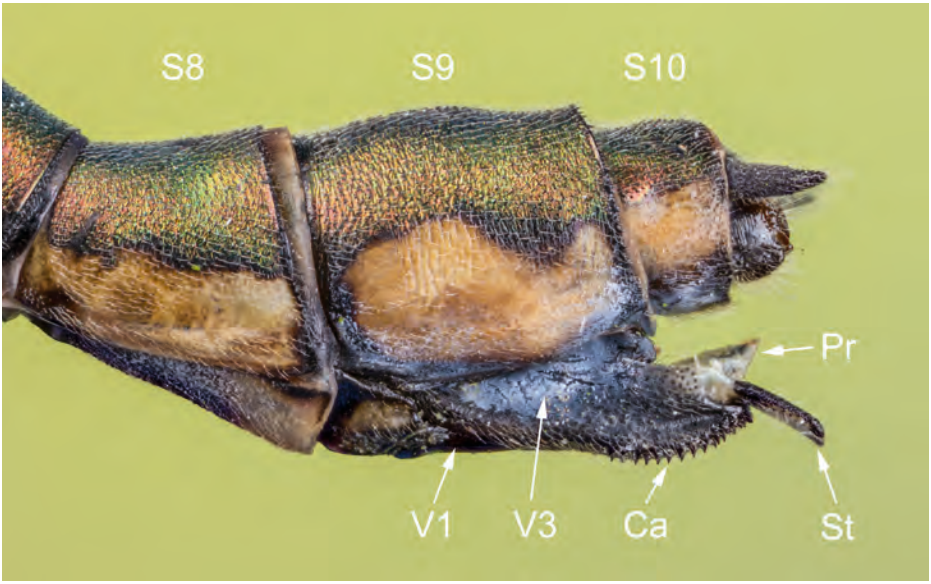
The ovipositor of *Chalcolestes viridis* is complex, requiring the ability to deposit eggs into woody plant tissue. During peak periods of activity, tandem pairs would frequently arrive at the lakeside *Salix* branches, and the structure of the ovipositor plays a significant role in sensing the most suitable place to start oviposition.

The paired cutting and sheathing valves of the ovipositor, combined with numerous sensory hairs on the styli, play a role is sensing the oviposition substrate (Plate 5A, B). When the styli make initial contact with the substrate they move over it to apparently determine its suitability. It was interesting to

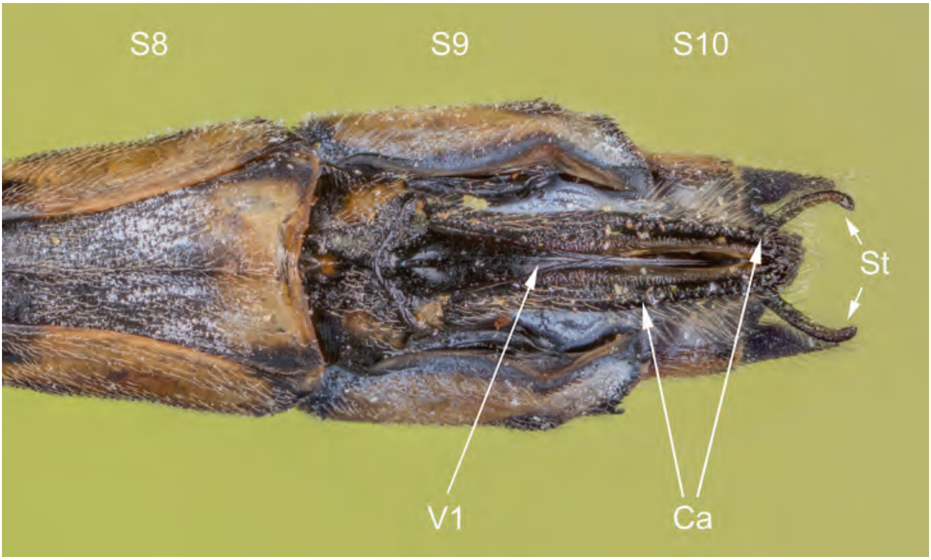
Plate 4. The study site at Stanford Lake (16 August 2020) showing *Salix* spp. (Willow) and *Lythrum salicaria* (Purple Loosestrife) growing around the lake margins. Branches of *Salix* close to the water were densely covered in oviposition scars (bottom insert). Branches further away had few if any scars (top insert).

note that, when the cutting valves penetrated deeper into the substrate or were withdrawn, the distance between the tips of the styli varied (Plate 6). Each time the female moved to the next insertion point the styli were used. Females were observed sensing the substrate and moving on further down the branch if any impediment for oviposition was encountered. In one oviposition line an obstacle had been detected and avoided by the female, with the egg set subsequently inserted to one side of the obstacle before continuing back along the original line (Plate 7). If a female in tandem did not start ovipositing after sensing the substrate the pair quickly moved to another branch. The lift off was well synchronised with the male able to sense that the female had not started ovipositing, presumably because it was an unsuitable site for oviposition.

The tips of the paired cutting valves are furnished with a series of saw-like teeth used to cut into the substrate (Plate 8). The cutting valves remain covered by the sheathing valves when not in use. Rows of carinae on the pair of sheathing valves make contact with the substrate once the cutting valves have partially penetrated the substrate. The sheathing valves bearing the rows of carinae appear to partly function with a ratchet-like function, maintaining contact with the substrate as the cutting valves penetrate deeper into the woody tissue and



A



B

Plate 5. The ovipositor of *Chalcolestes viridis*, showing abdominal segments S8, S9 and S10. (A) in side view; (B) in ventral view, where the full length of the cutting valve (V1) can be seen protected by the sheathing valve (V3) when not in use. Ca, carinae; Pr, projection on the sheathing valve; St, styli. The number of carinae are used to distinguish females from those of *C .parvidens*. Terminology after Matushkina & Lambret (2011).

Plate 6. A sequence of consecutive frames (A to D) taken from exactly the same viewpoint at one insertion point. It shows that, as the female lays a single egg, the distance between the tips of the styli (arrows) decreases as the depth of penetration increases. The vertical dotted line is positioned in the same place on each image to show the relative position of the styli and thus the penetration depth of the cutting valves.

Plate 7. Outer barks layers of *Salix* sp. showing the surface topography and insertion points (left) correlated with the underlying eggs (right).This pattern results from two oviposition events as the females moved down the stem. The arrow shows the obstacle where the female moved to one side to deposit an egg set before continuing on the original line.

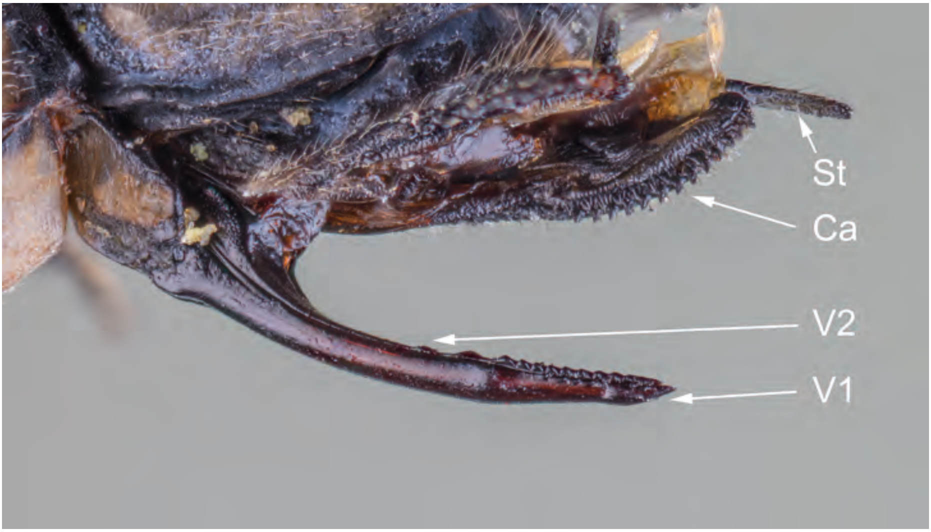


Plate 8. The ovipositor of C*halcolestes viridis* with the cutting valves moved out from the cover of the sheathing valve. V1, V2, the two parts of the cutting valves. Note the saw-like terminal end (V1) used to penetrate the oviposition substrate with a sawing action. Ca, carinae; St, stylus. Terminology after Matushkina & Lambret (2011).

Plate 9. Females of *Chalcolestes viridis* curve the abdomen strongly to exert the optimal force to penetrate the bark of *Salix* sp. The styli, sheathing and cutting valves of the ovipositor can be seen in contact with the substrate.

the cutting forces increase. This mechanism may also play a role in ensuring placement of the eggs at the correct depth. On one occasion, a tandem pair was interrupted during oviposition by a male *Sympetrum sanquineum* (Ruddy Darter), causing the tandem male to suddenly take flight. The male *C. viridis* tried to fly off, pulling the female away from the branch. The female became stuck in the *Salix* branch, hanging by just her ovipositor, preventing her from breaking free. She remained attached to the flying male at one end and to the branch by the ovipositor at the other for several seconds before she was able to free herself and fly off with the male. This incident indicates that the cutting surfaces of the teeth on the distal end of the cutting valves are backward facing, similar to that shown for other lestid species (Matushkina & Lambret, 2011). It is feasible that the forces exerted during the cutting action require the carinae to be in good contact with the substrate, enabling it to work against them to maintain the desired cutting direction.

Ovipositing females, either in tandem or alone, adopt a characteristic pose by bending their abdomens so the cutting valves of the ovipositor are located between the middle pair of legs (Plate 9). The joints between abdominal segments form right angles between S3 and S4 and between S4 and S5, enabling the ovipositor to be precisely positioned on the oviposition substrate. In almost all observations at Stanford Lake the ovipositor was between the middle legs during oviposition into *Salix* branches. On one occasion when a tandem female was observed ovipositing into the stem of *Lythrum salicaria*, the ovipositor was between the front and middle legs, suggesting that greater force was needed to penetrate the outer layers of this substrate. Initial observations also underestimated the use of *L. salicaria* for oviposition but further examination of the upright stems showed very indistinct markings suggestive of oviposition 0.4 - 0.6 m above the ground or water. Samples were taken and on dissection revealed numerous egg sets to be present yet with insignificant surface markings and no obvious oviposition scars (Plate 10). It appears that this plant is used for oviposition more often than realised and may go unobserved. Females of *Aeshna mixta* (Migrant Hawker) were also seen ovipositing into the same stems of *L. salicaria* as used by *C. viridis* but lower down, 0.05 - 0.3m above ground or water.

Oviposition: scars and egg density

In a single oviposition event, a female lays sets of eggs by making a linear series of incisions (an oviposition line) along the longitudinal axis of a branch of *Salix* sp. or other stiff woody plant stems. At each incision she inserts a set of between four and six eggs, at an angle to the substrate fibres in the outer layers of the bark tissue, either side of the insertion point (Plate 11). In the majority of oviposition events each incision results in an oviposition scar at the insertion point and a ‘blister-like’ raised mound over the egg sets beneath. As a result,

Plate 10. A stem of *Lythrum salicaria* (Purple Loosestrife) with oviposition 'scars'. The outer topography shows very indistinct insertion points (left), whereas removing the outer bark layers reveals the eggs (right).

the distinctive pattern is formed along the oviposition line. While the outer bark is still soft and the tree still actively growing, it is relatively easy to strip back the bark and expose the egg sets.

Egg sets from the Stanford Lake population typically comprised lines of four (2+2), five (2+3) or six (3+3) eggs (Plates 1 & 7). In some samples the egg sets showed eggs on one side and none on the other and some visible insertion points with no eggs. Egg sets with fewer eggs may reflect an interruption during oviposition. *Sympetrum sanguineum* was frequently observed in the same areas and seen on several occasions to disturb ovipositing tandems.

For a single uninterrupted oviposition line the gap between oviposition scars, measured from one edge to the next, ranged from 1.45 - 1.5 mm. For a single oviposition line there were typically 5 egg sets per cm, giving up to 30 eggs/cm^2. On some of the thinner branches with a diameter in the range of 6.0 - 6.5 mm the egg density was up to 44 eggs/cm^2 representing lines from three oviposition

Plate 11. Eggs are deposited in the outer layers of bark tissue of *Salix*, where they are well protected. Here the outer bark has been stripped away to reveal the position of two egg sets. The darker brown at the top shows the mound and insertion point.

events. For thicker branches with a diameter in the range of 9.0 - 9.5 mm the scar patterns had become merged and the egg density was up to 89 eggs/cm^2.

Along favoured branches, such as the larger diameter branches deeper in a *Salix* bush, the characteristic patterns became merged due to the high number of overlapping oviposition lines. In these areas the outer bark appeared to be darker as a result of the numerous eggs below the surface and possibly as a reaction by the plant to the presence of the eggs. Samples from these branches revealed numerous eggs, with many egg sets appearing to overlap with adjacent sets (Plate 12).

When the bark layer was stripped from thinner branches with a diameter of 6.2 mm the remaining heartwood had a diameter of 5.5 mm with the outer bark thickness being approximately 0.35 mm. Bark stripped from thick branches with a diameter of 9.2 - 9.4 mm had a remaining heartwood with a diameter of 7.9 - 8.0 mm. Here the outer bark had a thickness of 0.6 - 0.75 mm. Considering that the eggs of *C. viridis* are in the range of 1.275 - 1.3 mm in length and 0.275 - 0.3 mm in diameter at their widest point (Plate 13), the insertion of an egg or eggs into the outer layers of bark will create a significant increase in outer diameter, creating a mound that produces the characteristic raised pattern of

Plate 12. Outer barks layers of *Salix* showing the surface topography and insertion points (left) correlated with the underlying eggs (right).This pattern results from multiple oviposition events with no obvious pattern. The egg sets overlap in many areas.

the oviposition lines. The eggs lie at an angle to the plant fibres which hold them in place. There appears to be no abnormal growth by the plants cells around the eggs and therefore the mounds resulting from oviposition should not be described as galls.

Using the observed egg densities, the number of eggs per branch could be roughly calculated and thus the number of eggs per willow tree estimated. Multiplying this by the number of willow trees with oviposition scars at the site gives an estimation of the number of eggs deposited during 2020 to be in the hundreds of thousands. Considering the number of adults emerging each year it is clear that there is high mortality between egg and adult.

Detailed videos of oviposition (Cham 2021b for video link) showed the movement and angle of the female's ovipositor at each insertion point, signifying the number of eggs deposited (Plate 14). The female starts at each potential insertion point by touching the styli and cutting valves on to the substrate surface. At this point the ovipositor appears to vibrate at high frequency, which may serve to ease

Plate 13. Three eggs of *Chalcolestes viridis* dissected out of the outer bark layer. The darker end of the eggs (right) would be the end close to the opening of the oviposition scar.

the tip of the cutting valves into the woody substrate, in a similar fashion to a hammer drill. If found to be suitable, this is followed by the cutting valves penetrating the outer surface of the substrate perpendicular to the longitudinal direction of the branch using a thrusting action (Plate 14 i). Once inside the insertion point the ovipositor is rotated at an angle on one side with S8, S9 and S10 almost at right angles to S7, with the first egg then inserted in the hole at the top position (Plate 14 ii). The cutting valves are then partially removed and the process repeated at a slightly shallower angle to deposit the second egg (Plate 14 iii). A third egg may or may not be inserted at a shallower angle below the second. The cutting valves are again partially removed and S8, S9 and S10 rotated at an angle in the opposite direction so the process can be repeated on the other side of the insertion point (Plate 14 iv,14 v).

Videos showed that, after the last egg of each set is deposited, the cutting valves are extracted and the styli and carinae on the sheathing valves go through a series of rapid movements in close contact with the insertion point before moving to the next (Plate 14 vi). The reasons for this are not clear and it may function to modify the insertion point to ensure the opening is optimised in some way for future egg hatching. Observations of oviposition on algae-covered branches showed the carinae and other parts of the ovipositor to be covered

Plate 14. A sequence of a female laying a set of four eggs. (i) It starts with the female sensing the substrate surface with the styli, followed by penetration by the cutting valves, (ii) the ovipositor is moved at an angle on one side to deposit the first egg, (iii) the ovipositor moves to a slightly shallower angle to deposit the second egg, (iv) the ovipositor is moved to the other side at an angle to deposit the third egg followed by (v) the fourth egg. (vi) The process is finished by movements of the ovipositor to bring the styli and carinae into close contact with the insertion point, possibly preparing it for egg hatching the following year.

in debris, suggesting that the carinae teeth are abrading the surface and play a role in maintaining close contact with the substrate during the penetration of the outer bark layers. This abrasive action may cause a reaction from the plant that results in the characteristic oviposition scars at the insertion point. The carinae come into close contact as the cutting valves penetrate deeper into the bark layers and may act to provide more purchase on the substrate for the cutting forces to work against (Plate 15). The characteristic behaviour described indicated how many eggs were deposited in a set at each insertion point. Positioning the eggs in this way ensures that the eggs are held firmly in position at an angle by the plant fibres (Plate 16).

Oviposition rates were calculated from the video timelines by counting the number of eggs deposited at each insertion point. For instance, part of a sequence recorded two eggs in 31 seconds (i.e. 15.5 seconds/egg) and 4 eggs in 51.7 seconds (i.e. 12.95 seconds/egg). The longest sequence was recorded for 29 minutes 59 seconds and, over this period, 18 egg sets comprising a total of 70 eggs were deposited. This equates to 2.41 eggs/minute (24.9 seconds/egg). Of these egg sets, 13 out of 18 had a 2 + 2, two had a 2 + 3 and two had a 2 + 1 set. At the start of the video only the second half of the first insertion was recorded, with two eggs observed being deposited. Of the 18 egg sets, 11 started on the female’s left side and seven on the right. There was no obvious pattern to the side at which each egg set started in the sequence. During this recording the female was distracted several times by other tandem pairs arriving, being buzzed by a male *Sympetrum sanguineum* and a fly landing close by. The variability in the size of egg sets during any one oviposition event suggests that the female is actively sensing the suitability of the substrate for egg deposition and modifying her egg set at each insertion point.

Small sections of branches where oviposition scars had been observed in August were taken on 8 October 2020, nearly six weeks after peak oviposition activity. Close examination showed the insertion points along the oviposition line to have opened up as a result of the continued growth of the branch, forming extensive scars. Under the microscope the remains of withered eggs could be seen on the surface, partially held in the area of former openings (Plate 17). Dissection of some of the mounds revealed darkened and collapsed eggs in the process of decomposing. Viable eggs showed development of egg yolk globules inside the eggs. This would suggest that the continued growth of *Salix* branches appears to be a factor in determining successful egg development.

Discussion

The genus *Chalcolestes* is represented in Europe by two closely related species;

Plate 15. As the cutting valves of the ovipositor penetrate deeper into the woody tissue the carinae maintain good contact with the substrate. Ca, carinae; St, styli; V, cutting valves.

Plate 16. The thin outer layer of the bark of *Salix* sp. has been removed to reveal the eggs. The eggs are positioned at an angle to the plant fibres in the outer layers of the bark. This orientation may give extra security to the eggs.

Plate 17. An oviposition line showing extensive scarring as the *Salix* branch continues to grow. The remains of eggs can be seen partially held in the position of the original insertion points (arrows). The inset shows a close-up of an egg that has been exposed through growth of the wood and expansion of the oviposition scar.

Chalcolestes viridis (Western Willow Spreadwing - Willow Emerald Damselfly in the UK) and *C. parvidens* (Eastern Willow Spreadwing). *Chalcolestes viridis* is a common and widespread species across much of continental Europe and is relatively well studied (Münchberg, 1933; De Block & Stoks, 2005; De Block *et al*., 2005; Schiel & Buchwald, 2015).

European field guides (Dijkstra & Lewington, 2006; Dijkstra *et al*., 2020; Smallshire & Swash, 2020) use the number of teeth (carinae) on the ovipositor to differentiate females of *C. viridis* (10-14 teeth) from those of *C. parvidens* (6-8 teeth). The numbers quoted in these guides are for one side only, which can initially be misleading as the rows of carinae are present on both sides of the ovipositor. Thus *C. viridis* has a total of 20-28 carinae on the ovipositor (Plate 5). The difference in number of carinae between the two species, which were formerly regarded as subspecies, suggests a difference in the utilisation of oviposition substrates. The ecological differences for the two *Chalcolestes* species are poorly known (Dijkstra & Lewington, 2006) and having more carinae could give *C. viridis* an advantage to utilise a wider stiffness range of plant substrates than *C. parvidens*. This may help to explain the much wider distribution and abundance of *C. viridis* across Europe.

Peak activity and oviposition take place in the afternoon at Stanford Lake, which concurs with Dijkstra & Lewington (2006) who noted that, in Italy, most activity of *C. parvidens* is in the morning, whereas for *C. viridis* it is in the afternoon. Riley (2020) also states that *C. viridis* does not become fully active until late morning onwards.

Matushkina & Gorb (2007) assessed the mechanical strength of damselfly ovipositors, which included *C. parvidens,* and their preferred plant substrates. They concluded that the degree of curvature of the abdomen differed between species and, during oviposition, correlated with the substrate stiffness and therefore the force required for the ovipositor to penetrate the substrate. Out of 18 plant species used for oviposition, *Salix* sp. was the stiffest, requiring greater force to penetrate. They showed a significant positive correlation between the bending stiffness of the ovipositor and plant substrate stiffness. They also demonstrated statistically significant differences in ovipositor stiffness, with the studied species arranged in the following order according to ascending stiffness: *Calopteryx splendens* (Banded Demoiselle), *Sympecma annulate*, *Lestes virens* (Small Spreadwing)*, Coenagrion pulchellum* (Variable Damselfly)*, Lestes sponsa*, *Lestes barbarus* (Migrant Spreadwing) and *C. parvidens*. *Chalcolestes viridis* would be expected to have a similar or higher bending stiffness to that of *C. parvidens* on this scale.

Matushkina & Gorb (2000) reported that eggs are oviposited in complex linear chains, with up to eight eggs in one perforation, and suggested that the formation of an egg-laying ‘chain’ with clear parameters (i.e. the distance between insertion points, exact orientation of the egg set and individual eggs in it in relation to the substrate fibres) reduces the likelihood of damage to previously laid eggs. Egg sets of four to six eggs were the norm in the samples examined from Stanford Lake. Egg sets of eight were not found at Stanford Lake, nor were four eggs recorded on any side.

Matushkina & Gorb (2000) noted that the maximum egg-laying density of *C. viridis* was 29 eggs/cm^2 of substrate. At Stanford Lake, there were about 30 eggs/cm^2 in a single oviposition line and the egg densities observed here significantly exceeded those reported by Matushkina & Gorb (2000), i.e. more egg sets yet with fewer eggs in each. At high population densities the demand for oviposition substrate increases, leading to multiple egg set insertions along branches. Plate 12 clearly shows a complex of oviposition scars and oviposition lines made by multiple female oviposition events. In some samples the bark around the insertion points was darkening, suggesting a reaction from the plant tissue. It is also surprising that tandem pairs still continued to arrive and commence oviposition on branches already covered by prominent oviposition scars. Matushkina & Gorb (2000) also suggested that the formation of an egg laying ’chain‘ with clear parameters reduces the likelihood of damage to previously laid eggs. The results presented here show that egg overlap was frequent on favoured branches of *Salix* bushes. Whether this incurs any damage to existing eggs was not investigated. The correlative lighting method has proved to be a useful tool to assess egg set size and positioning in substrate samples and it would be interesting to look at other populations in the UK using this technique and also to see if egg set size and patterning is related to the oviposition substrate. It is relatively easy to remove the new growth bark from *Salix* spp to assess oviposition lines for egg sets but not so easy as the plant ages and the outer bark hardens.

The terminology used in recent guides has been inconsistent with both ‘gall’ and ‘scar’ used for the raised area at each insertion site resulting from oviposition. A gall is defined by the British Plant Gall Society as an abnormal growth produced by a plant or other host under the influence of another organism (Redfern & Shirley, 2011). It involves enlargement and/or proliferation of host cells, and provides both shelter and food or nutrients for the invading organism. Whilst the eggs of *C .viridis* are protected in the outer layers of bark (Plate 11) there appears to be no abnormal growth, enlargement or proliferation of the host cells and the blister-like mounds along the oviposition line result from the physical upward displacement of bark tissue by the egg sets (Plate 16). Scars are defined as any blemish remaining as a trace of, or resulting from, injury or use. In the case of *C. viridis*, scars result from the process of oviposition rather than the presence of eggs and should therefore be referred to as oviposition scars rather than egg scars. It is postulated that the scarring could result from the abrasive action of the ovipositor’s carinae during oviposition. It has also been shown in this study that oviposition scars at insertion points can expand on branches that are still actively growing, resulting in exposure and destruction of eggs (Plate 17). This also results in egg sets becoming more exposed to external factors, with some egg sets in areas of extensive scarring showing signs of decomposition. The sequence of egg sets along the oviposition line has also been referred to as both egg or oviposition ‘tracts’ and ‘track-like scars’. ‘Tracts’ are defined as large areas of land or refer to a major passage in a body, such as the digestive tract, and therefore is inappropriate terminology in the context used here. ‘Track’ is more acceptable, referring to the marks left by an animal, person or vehicle in passing.

Various structures of the ovipositor, including the styli, are well endowed with sensory organs that provide information and control of the relative movements of the ovipositor’s mechanical system, contact with the substrate and relative positioning of the ovipositor to the substrate. Whilst the morphology of the ovipositor has been well studied in *Lestes macrostigma* (Dark Spreadwing), with the use of SEM revealing numerous sensory hairs and pits covering the various parts of the ovipositor (Matushkina & Lambret, 2011), the exact function of the styli during oviposition is less well understood. Matushkina & Gorb (2002) have shown that the surgical removal of one stylus (or both styli) in *L. sponsa* resulted in a decrease in the maximum angle of eggs and asymmetry of egg positioning, with a shift of the oviposition line to the side with the intact stylus. They concluded that the mechanosensory system of styli in *L. sponsa* is involved in the oviposition line and in egg positioning in the egg set, which may serve to reduce damage when ovipositing in a stem containing previously deposited eggs. Close up images (Plate 6) and video sequences revealed that, as the cutting valves penetrate the woody substrate, the distance between the two tips of the styli decreases as the depth of penetration increases. The reason(s) for this are not clear.

The time taken to oviposit is reported to be slow in *C. viridis* compared to other lestid species, at around one minute/egg (Matushkina & Gorb, 2000). It is considered that they are at risk of predation during this time and therefore contact guarding by males during oviposition has advantages. At Stanford Lake, single females were observed ovipositing alone in the evenings and it might be that the predation threat has diminished at this time, since the time taken to deposit each egg ranged from 12.95 - 24.9 seconds per egg, which is considerably shorter than that noted by Matushkina & Gorb (2000).

The ability of *C. viridis* to oviposit into substrates such as woody tree bark provides a defence for their eggs, thus increasing the chances of offspring survival compared to closely related species such as *L. sponsa* that oviposit into soft-stemmed herbs. In the latter, the eggs are potentially more exposed to parasites. Harabiš *et al*. (2019) found there was a significantly higher proportion of parasitized eggs for *L. sponsa* (4.7%) compared to *C. viridis* (1.0%). Their results showed a significantly higher prevalence of parasitoids from the genus *Aprostocetus* (a species of eulophid wasp) in *L. sponsa* than in *C. viridis*. This would suggest that parasitoid species are somehow limited in their ability to detect and/or attack *C.viridis* eggs and they concluded this is because *C. viridis* eggs, which are generally oviposited into stiff tissues, are better protected against predation and parasitoids. Another possible explanation is that *C.viridis* tends to oviposit into the bark of trees, which are usually several metres above the aquatic habitat, and thus at the edge of interest for parasitoids looking for aquatic hosts. During the current study there were no observations that would suggest parasitoids to be present. As a relatively new arrival to Britain, *C. viridis* has the opportunity to colonise new sites where suitable oviposition sites are available. They may also have the advantage of being relatively free from parasitism since two of their known hymenopteran parasitoids in Europe, *Aprostocetus pseudopodiellus* and *Prestwichia aquatica* (Harabis *et al*., 2019), have so far not been recorded in the UK (NBN, 2020), although it is possible that existing resident parasitoids may adapt to using *C. viridis* as a host.

Where suitable oviposition substrate is present new populations can arise relatively quickly. However, population size appears to vary significantly and some sites do not have the same high numbers after several years as recorded at Stanford Lake (*pers. obs.*) During 2020 *C. viridis* was especially abundant at Stanford Lake but this was not the case at other sites in Bedfordshire where it was also recorded in previous years. *Salix* spp are a common and widespread group of tree species associated with wetland habitat across the UK, and further spread of *C. viridis* is expected to colonise new areas in the future. Early colonists tend to exhibit boom-bust dynamics (Strayer *et al*., 2017) with new populations declining after a few years. The UK has experienced other new colonists in recent decades and, following the arrival of *Erythromma viridulum*, new sites were initially colonised in high numbers followed by a decline (A. Parr *pers.com*.; *pers. obs.*). Similar observations to this have been made for *C. viridis* in Suffolk (A. Parr *pers.com.*).

Chalcolestes viridis is expanding its range across Britain, a trend that looks set to continue. The new population at Stanford Lake has grown to a significant level in one season and it will be useful to monitor the site in future years to assess if the high numbers are sustainable. *Chalcolestes viridis* appears to be highly dispersive, moving to new sites across Britain, and it would be interesting to find out at what point there is a mechanism to trigger dispersal to fresh areas.

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Egg hatching, prolarvae and larval development time of *Chalcolestes viridis* (Vander Linden) (Willow Emerald Damselfly) in Britain.

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Summary

Chalcolestes viridis (Vander Linden, 1825) (Willow Emerald Damselfly) was first recorded in Britain in 2007. The female behaviour of ovipositing into the branches of willows and other woody plant species is different to any other British species with endophytic oviposition. Egg hatching and larval development times have so far not been studied in UK populations and are the subject of this paper. The results of the study indicate that the hatching period for eggs of *Chalcolestes viridis* is between seven and nine days. Eggs predominantly hatched at night or by 06.00 BST. The viable prolarval stage, when out of water, could last for at least 1 hour 30 minutes. The distance prolarvae propel themselves could be at least 50 mm, confirming the observations of other researchers. The second stadia larvae, which are 3 mm long at transition, need to be on a horizontal water surface to extricate themselves from the prolarval sheath. Observations show the sheath to be composed of hydrophobic droplets, which assist this process.

Introduction

Chalcolestes viridis (Willow Emerald Damselfly) was first recorded in Britain at Trimley near Felixstowe, Suffolk in 2007 and has since spread significantly across many areas of Britain (Cham *et al*., 2014). Emergence of adults has been recorded from mid-June with a peak of adult activity in mid to late August and the last individuals still on the wing in mid-November. This is similar to the flight period in central Europe, where it also starts to emerge in mid-June, with the main activity period in August and the last individual usually seen at the end of October (Dolný *et al*., 2016).

Oviposition by *C. viridis* typically proceeds in tandem immediately after copulation and occasionally alone, with eggs inserted into stiff substrates, primarily the bark of softwood trees. A wide range of plant species is used for ovipositing, including *Salix* (Willows), *Populus* (Poplars) and *Alnus* (Alders) (D'Aguilar *et al*.,1986; Parr 2016) and Martens (1997) noted the species ovipositing in the conifer *Pinus sylvestris*. It is considered that female *C. viridis* have to expend significant energy to penetrate these woody substrates to deposit their eggs. Therefore, their oviposition rate is slow compared to other damselfly species (Matushkina & Gorb, 2000). Eggs are inserted into the outer layers of the bark of branches and stems, which results in a small opening at each insertion point with a set of eggs deposited at an angle on either side. The egg set size is variable and ranges from 4-6 eggs in Britain (Cham, 2021a) and up to eight (four either side) in Europe (Matushkina & Gorb, 2000).

The insertion of eggs causes the outer layer of the plant tissue to rise up, forming a slight blister-like mound over the eggs, which results in a characteristic linear pattern of oviposition scars. The terminology in the literature is confusing and has been discussed by Cham (2021a), who proposed that these mounds should be referred to as oviposition scars. The egg is the over-wintering stage of this univoltine species and the hatching of the eggs is reported to occur during the following April and May in continental Europe, followed by rapid larval development (Schiel, & Buchwald, 2015). To date, little is known about the egg hatching process and the duration of larval development in Britain (Parr 2016).

The hatching of zygopteran eggs generally, consists of three stages (Corbet, 1999). Firstly the embryo swallows amniotic fluid, resulting in an increase in pressure that ruptures the endochorion. Secondly, the embryo swallows water causing the vitelline membrane to burst and the prolarva to slide out. Thirdly, the active intake of water enables the prolarval cuticle to split. The sequence in *C. viridis*, where eggs hatch without being wetted (Pierre, 1904) is significantly different, hence Corbet (1999) considered that *C. viridis* was one of only several odonate species where the first instar could be regarded as a 'true prolarva', that can reach the water by jumping (leaping) or springing after completely separating from the egg. In contrast to other odonates the egg hatching mechanism of *C.viridis* is difficult to observe due to the eggs being located in woody or hard plant tissue. Attempts to dissect around the oviposition openings to expose the eggs prior to hatching is problematic and can result in irreversible damage to the eggs. The outer woody tissue of some plant species does not freely separate from the twig as it ages and becomes woodier.

Adults of *C. viridis* have been recorded in new areas across central Bedfordshire during August and September 2019. The species was recorded for the first time at Stanford Lake (TL159407), 2km north east of Shefford. This is a well-recorded lake in a small area of woodland, managed by the Forestry Commission, with no previous sightings of *C. viridis*. A fishing syndicate has previously used the lake but, following many years of low water levels, the fishing interest has diminished. During September 2019 there were many tandem pairs ovipositing into the branches of *Salix spp* (Willows) and *Salix caprea* (Sallow or Goat Willow) around the margins of the lake. This resulted in numerous distinctive lines of oviposition scars along the branches of a number of these trees. This discovery in 2019 provided the opportunity to study egg hatching in the following spring and to compare the process with that described by Pierre (1904) over one hundred years ago.

Abbé Pierre (1904), in a classic study, was the first to discover and describe the prolarval stage of odonates while investigating the hatching of eggs of *Lestes* (now *Chalcolestes*) *viridis* from 'galls' formed on the branches of the willow *Salix aurita*. He documented the process of eggs hatching and how the prolarvae drop onto the surface of the pool below. Prolarvae that landed on the ground were seen to jump (leap) around until they reached water. He described the prolarva enclosed in a sheath, which he considered to be armoured all over with small overlapping plates, like a coat of chain mail, and noted that the prolarva jumped clear of the stem from where it hatched by strong bending movements of its body, falling into the water below. Corbet (1962) noted that the longest recorded jump by *C. viridis* prolarvae is 3 cm. Prolarvae were recorded to be active for up to two hours on the ground and, when they finally landed on water, they rested immobile on the surface. The prolarvae floated with their dorsal surface downward before the second stadia larvae emerged. Ecdysis was reported to last 13 minutes before the second instar larvae passed directly into the water below (Pierre, 1904). Of particular note, the cuticle was regarded to be hydrophobic to prevent the prolarvae being imprisoned by drops of water encountered en route to the pool (Corbet, 1962). This also serves as a potential mechanism to prevent premature moulting in pockets of water or rain droplets and thus to postpone ecdysis until the prolarvae are immobile on a horizontal water surface.

Methods

Branches with extensive lines of oviposition scars were cut with sharp secateurs from *Salix caprea* and two other willows at Stanford Lake in October 2019. The base of each branch was placed in a bucket of rainwater within one hour of collection to promote continued growth of the plants, which typically root easily. The branches were maintained like this outdoors over the winter period. Signs of budding and leaf growth were evident in February and March 2020, confirming the viability and continued growth of the plants.

During early April 2020 improving weather was forecast after a cool wet period, suggesting that conditions might be suitable for egg hatching. Egg hatching would be very difficult to observe outside so, in anticipation of this difficulty, a selection of ten branches covered with oviposition scars were cut into manageable lengths of approximately 20 cm and brought indoors. Each length was initially inspected under a stereomicroscope using twin LED lighting at between 10 to 64x magnification for signs of eggs in the openings of the oviposition scars. Each was then held individually in the centre of a large ceramic ‘coffee’ mug (100 or 110 mm in diameter) containing 2 cm depth of rainwater, using a length of green plastic coated garden wire to hold the branch upright (vertical) (Plate 1), to allow any hatching larvae to ‘fall’ or ‘crawl’ into the water below. Each container was placed on a south-facing windowsill. Over the period from 7 - 26 April high pressure over southeast England brought predominantly warm sunny weather. From 7 April onwards the branches were monitored daily for signs of egg hatching. The maximum daily room temperature was recorded using a digital room thermostat. Containers were checked regularly throughout the day from 06.00 to 22.30 BST.

Plate 1: A *Salix* branch held in the centre of a water-filled container on a window sill.

Six shorter lengths of branch (~15 cm) with oviposition scars were cut and completely submerged in pond water in containers to assess the potential for eggs hatching underwater. These were left for seven days in water (to mimic flood conditions) before being removed and held in containers as described above. Another six branches were cut and left hanging in air over water to mimic completely dry conditions, as would be the case with damaged branches.

Sections of other branches were cut at random at various times and brought inside for dissection of oviposition scars, to assess the status of eggs and/or egg remains. These were monitored for further hatching but were not used for daily counts. Tweezers with ultra-fine tips (Dumont type N5) were used in combination with a fine surgical scalpel to minimise any impact of the dissection process on the eggs.

Once hatching was confirmed and numbers recorded, the resulting prolarvae (1st stadia) and larvae (2nd stadia) were observed under a stereomicroscope in a small water-filled container using a micrometer scale to measure their length. Each stage of the process was photographed and recorded on video using a Canon M6 mkII digital camera mounted on the microscope. After inspection and counting, the contents of the containers with 2nd stadia larvae were released into the author's pond and the container replenished with fresh pond or rainwater. The release of larvae was in anticipation of determining the duration of larval development to adult emergence over the subsequent months, under close to natural conditions in the pond.

During late June and throughout July 2020, regular visits were made to Stanford Lake to check for the first signs of emergence and teneral adults. Visits were also made during August 2020, when adult numbers were high and ovipositing activity was at its peak (Cham, 2021a) to record any subsequent changes to the oviposition scars. The garden pond was also checked daily for any signs of emergence.

Observations

On initial inspection, the selected branches showed 'openings' of varying sizes in the areas with the distinctive pattern of oviposition scars. Some were open and others partially or completely covered with woody material. Inspection under the microscope revealed the tips of unhatched eggs in many of the openings. Eggs were relatively easy to locate from their shiny surface reflection, which was noticeably different to that of the surrounding woody material (Plate 2).

Small 2nd stadia larvae, measuring approximately 3 mm from the front of the head to the tip of the caudal lamellae (Plate 3), were present in two of the containers at 07.30 on 12 April 2020, indicating that the first egg hatching had occurred during the previous night or early hours of the morning. The room temperature during the preceding day had been the warmest day of the year to date, reaching a daytime peak of 25°C (Table 1).

The following morning (13 April) revealed more newly hatched larvae (estimated at >50) in the same two containers. This was a cooler day with temperatures in the room at 21°C. On 14 April newly hatched larvae (estimated at >80) were present in the two containers. It was also noted that cast prolarval sheaths were visible on the water's surface. At 7.30 on 15 April approximately 30 2nd stadia larvae were present. The count at 6.30 on 16 April, recorded 21 2nd stadia larvae and there were only eight and one on the next two days (Table 1). No further hatching was observed in these containers.

Although other containers had larvae, due to time constraints these were noted but not counted. Of the branches brought inside for dissection of oviposition scars on 16 April there were no larvae on the following day yet there were larvae present on 18 April during a wet overcast day outside and with an inside room temperature of 17.5°C. Egg hatching from these branches slowed down

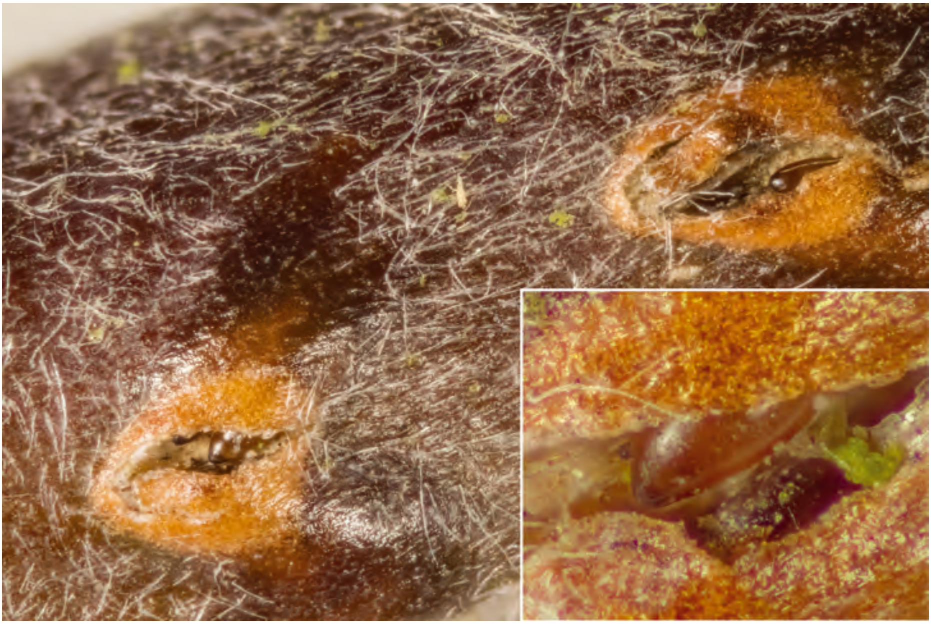

Plate 2. The tips of eggs of *Chalcolestes viridis* visible in the openings of oviposition scars. Inset shows magnified view of one of the egg tips.

Plate 3. A 2nd instar larva of *Chalcolestes viridis* showing successful hatching from the branch samples.

Table 1. Daily count of 2nd stadia larvae in two containers in 2020. No larvae were recorded after 18 April.

Date	Number of 2nd stadia larvae	Maximum daily room temperature (ºC)
11 April	0	25.0
12 April	15	23.5
13 April	>50	21.0
14 April	>80	22.0
15 April	~30	21.0
16 April	21	18.5
17 April	8	19.0
18 April	1	17.5
19 April	0	

significantly by 23 April. A few eggs were still hatching on 25 April with only one 2nd stadium larva observed on 27 April. On 28 April and again on 29 April there were no new hatchings, suggesting that the majority of eggs had either hatched at this point or were not viable and had perished.

During the 2019/2020 winter period the Forestry Commission cleared many of the willows that had oviposition scars containing eggs from the north margin at Stanford Lake. One of the willows from which branches had been taken survived on a small island in the lake. However, many other willows remained around the other margins of the lake. On 19 July 2020 the first teneral male was observed flying up from the lake margin, confirming emergence and successful breeding at the site from the eggs laid in 2019. On subsequent visits more teneral adults and exuviae were recorded, with numbers of adults building up significantly in early August (Cham, 2021a).

The egg hatching process

Unlike other species of damselfly, where egg development and hatching can more readily be observed, the eggs of *Chalcolestes viridis* remain covered by dense woody material in their oviposition scars. This makes the hatching process difficult to predict and observe, as there are no advance indications of which eggs are about to hatch and from which opening.

The hatching process starts when the prolarva breaks free, head first, from the anterior end of the egg, leaving the eggshell remaining in the oviposition

Plate 4. Extracts from a video sequence showing the prolarva leaving the opening of the oviposition scar. (i) the prolarva exits the opening and maintains close contact with the branch, (ii) and (iii) the frontal section of the prolarva starts to bend and arch away while still in contact by the hind section, (iv) the prolarva curls in readiness to propel itself away from the branch.

scar. The emerging prolarva has a distinctly dark area at the frontal part of the head (Plate 4 i - iv.). This is the egg burster or *ruptor ovi*, which is present in the prolarva of a number of odonate species (Corbet, 1999) and is a sclerotised crest that is used to rupture the chorion of the egg.

A prolarva could suddenly emerge out of any of the many oviposition scars. As most of the hatching occurred during the night or early hours of the morning this was especially difficult to observe. However, prolarvae were observed at the point of exiting on five occasions and one hatching sequence was recorded on video at 09.25 on 13 April when one was exiting its oviposition scar (Cham 2021b for video link). Observations supported by analysis of the individual video frames (Plate 4 i-iv) and still photographs showed the hatching to proceed as follows:

The emerging prolarva is encapsulated in a sheath as it separates from the egg. It is this stage that has been considered as a true prolarva (Corbet & Brooks, 2008). The sheath is transparent and encapsulates all parts of the body, including the legs, and is covered with a thin coating of liquid, presumably amniotic fluid from the contents of the egg (Plate 4 i.). The liquid coating appears to ease the process of leaving the oviposition scar, enabling the prolarva to exit the scar opening. As it moves away from the opening it does not immediately drop, but stays in close contact with the twig, using the surface tension of the liquid. Initially, the prolarva is stretched out and moves with a maggot-like movement where the muscles in the abdominal segments contract in forward-moving waves to produce a series of telescoping peristaltic movements (Berrigan & Pepin, 1995). Once the prolarva is clear of the opening it exhibits curling and arching of the body with only the last few of its distal segments remaining in close contact with the branch (Plate 4 ii., iii.,iv.). In less than 40 msec it propels itself by leaping to get clear of the branch. Thus analysis of the video running in 4K at 25 frames/second showed the moment of leaping to be too fast to be recorded in detail, with adjacent video frames showing the prolarva to be present in one frame and gone in the next. This prolarva leaped horizontally through the air, landing on the flat, hard plastic sheet used on the microscope, a distance of approximately 3 cm from the branch. It was then transferred to a small glass chamber containing rainwater for further observation.

Having observed the leaping mechanism several times, the windowsill in the area surrounding the study containers was inspected and to my chagrin more prolarvae were discovered out of water. These had propelled themselves away from the twigs from where they had exited the oviposition scars, landing outside the water container, a distance of at least 5 cm. They were observed springing around as described by Pierre (1904) and further encouraged by gently blowing over them. Each one was collected on a fine brush or in a drop of water from a small plastic pipette and placed in rainwater in the observation chamber under the microscope. These prolarvae had been out of water for at least 1 hour 30 minutes and all were able to recover in water and go on to the 2nd stadium. Observations showed the 1st stadia prolarvae to still be in their sheath and floating on the surface of the water (Plate 5a). Under the microscope, the prolarvae were initially observed making rocking motions in an attempt to free themselves from the sheath, which is comprised of many spherical ‘fat-like’ droplets (Plate 5b). This equates to what has previously been described as “delicate chain mail, with a very fine scaly appearance arranged in a series of

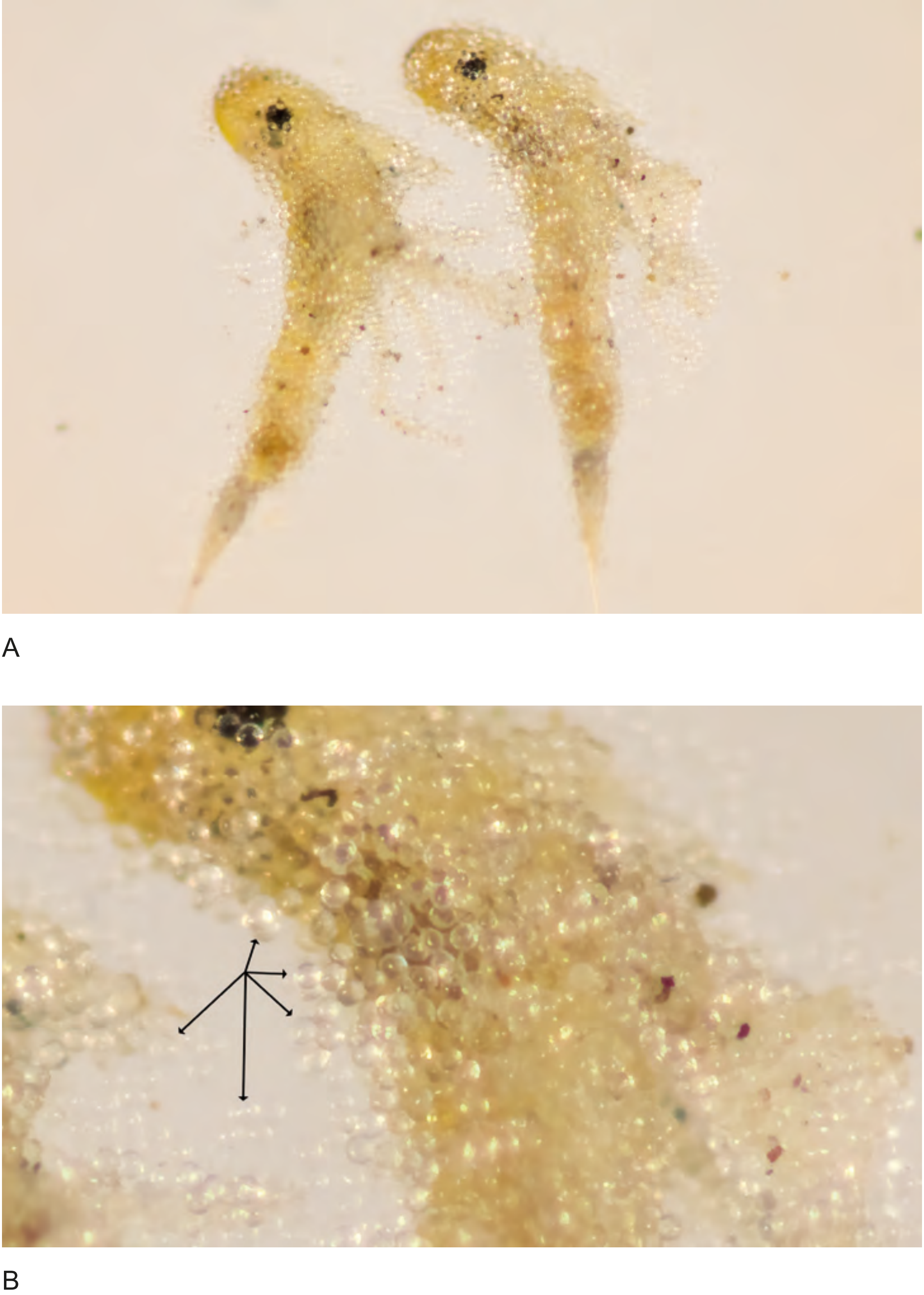

Plate 5. (A) Two prolarvae float on the surface of the water as they try to free themselves from the sheath which is covered in hydrophobic droplets, (B) Magnified view to show the hydrophobic liquid droplets (arrows) of the prolarva sheath.

Plate 6. A prolarva in the final stage of freeing itself from the sheath, which remains floating on the water surface. Note the remaining droplets on its head that cause it to stay in contact with the water surface.

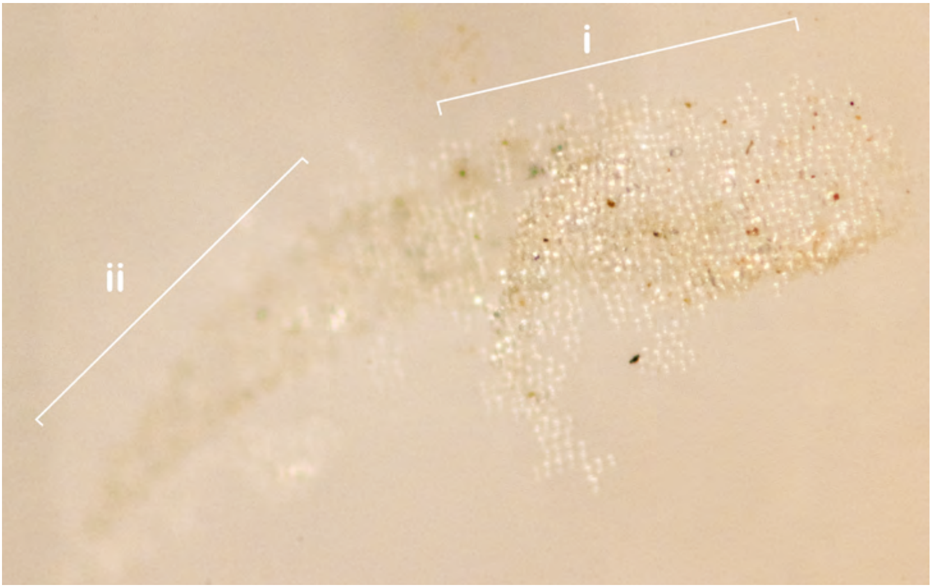

Plate 7. The remains of the sheath floating after the 2nd instar larva has freed itself. The hydophobic droplets (i) remain on the surface while the rest of the sheath (ii) starts to sink.

belts” (Pierre, 1904) or armoured all over with small overlapping plates (Tillyard, 1917). Larvae were able to free themselves from the sheath with their dorsal surface pointing downwards and then started to move around in the water. The remains of the sheath and droplets initially stayed floating on the water as the larvae broke free (Plate 6). The droplets continued to float on the surface and slowly disperse, while parts of the prolarval sheath started to sink (Plate 7). Attempts were made to try to get the sheath and droplets to sink by prodding with a mounted needle, but they remained floating. These observations indicate that the droplets have strong hydrophobic properties that appear to play a significant role in keeping the prolarvae on the water surface during ecdysis.

No hatching was observed, or prolarvae found, at any time in the containers where the branches had been completely submerged in water. Furthermore, no hatching or larvae were found after these twigs had been removed and kept with just their bases submerged in water. Similarly, the twigs held under dry conditions also failed to show hatching at any time. In each case it is assumed that any viable eggs had perished as a result of submergence or drying respectively.

A range of oviposition scars, including those with an obvious opening and those with none, were dissected as carefully as possible to reveal any eggs or their remains. This proved to be particularly difficult as the woody outer layers of the twig had hardened over the winter period and were resistant to the cutting actions of the scalpel and movement by the tweezers. Of those cut open, some showed signs of eggs having successfully hatched. Other scars had shrivelled eggs, which initially looked as though they had hatched, but close inspection at high magnification revealed the darker parts of dead and shrivelled prolarvae. The heavily pigmented eyespots visible in prolarvae were noticeable in these shrivelled eggs when viewed under the microscope.

During visits to Stanford Lake in August 2020, fresh growth branches with new oviposition scars showed clearly discernible holes one month after the eggs had been deposited and when no eggs would have hatched (Plate 8A). Actively growing branches with oviposition scars from the previous year showed signs of stretching of the openings as the girth of the growing branch increased, creating a larger opening than the one created at the time of oviposition (Plate 8B). In both cases the presence and/or the size of the openings does not represent a reliable means to determine successful hatching of eggs.

In the early days of monitoring, small fragments of woody material were observed in one of the containers, which appeared to be dropping into the water from the branch sample. Further investigation revealed small mites living in some of the openings formed by the oviposition scars. Small white mites could be seen

Plate 8. Oviposition scars on *Salix* (A) new growth of *Salix* four weeks after oviposition, showing distinctive openings and before any eggs would have hatched, (B) Openings from previous year's oviposition scars, showing the effect of stretching resulting from the continued growth of *Salix*.

which were presumed to be immature. Larger adult mites were red-brown in colouration and could be found clustering in some of the openings and cracks in the outer layers of the twigs. In some openings, orange coloured, oval-shaped eggs, presumably of mites, could also be seen. The mites were observed on a number of the twigs but their impact on the development and hatching of *C. viridis* eggs was not investigated. There appeared to be no indication of eggs being predated by the mites but they may have some impact by retarding or preventing egg development and hatching.

Discussion

Until recently little has been known of hatching time and larval development time of *Chalcolestes viridis* in Britain. Being an obligatory univoltine species, *C. viridis* over- winters in the egg stage and has a short aquatic larval development stage in Europe of about three months after the eggs hatch in spring. A study under 'semi-natural' conditions in southwest Germany (Schiel & Buchwald, 2015), reported the first eggs starting to hatch at the end of March, with some hatching continuing through to the end of May (median date: 15 April). Similar dates have also been reported for other central/north-western European localities (e.g. Münchberg, 1933; De Block & Stoks, 2005; De Block *et al*., 2005; Schiel & Buchwald, 2015). In Belgium, De Block and Stoks (2005) found a total of 1425 eggs hatched over a period of six weeks (7 April–21 May). A. Parr (unpub. obs.) examined oviposition scars at Alton Water, Suffolk, on 16 April 2012, that showed signs of significant hatching. The timing of egg hatching observed in this study fits with his observations.

Pierre (1904), who also recorded hatching in the first two weeks of April, suggested that hatching and exit is shortest when conditions are warm and humid. Indeed, the first hatching observed in this study occurred at night after the days when the April daytime indoor room temperature reached 25°C and concluded after 7-8 days. Corbet (1999) discussed diel periodicity of hatching in Odonata, which serves to confine hatching to a time that favours survival of the prolarva. It is regarded that the prolarva is the first stadium of *C. viridis* (Corbet & Brooks, 2008) and there are typically 10 stadia (or instars) during larval development (Schiel & Buchwald, 2015), although Münchberg (1933) reported as many as 13 stadia. Peaks of hatching observed in this study occurred during the night and before 06.00. This is a time period when prolarvae landing on dry ground are potentially at least risk of desiccation and predation.

Based on the timing of egg hatching in April observed in this study, with the first emergent adults observed at Stanford lake in July, a larval development time of approximately 90-100 days would fit with previously published times. Reports of a roughly 60–70 day developmental period are widely mentioned elsewhere in the literature (e.g. Münchberg, 1933; De Block & Stocks, 2005), though Robert (1959) reported a value of just over 100 days. In southwest Germany, in a detailed laboratory study at ambient temperatures, Schiel & Buchwald (2015) found full larval development to take on average 59 days, with a range of 53–62 days. The possibility remains that bringing branches inside, as in the present study, just before hatching was expected, triggered the hatching ahead of those under natural conditions. It is also likely that adult emergence in the preceding days in July had been missed at Stanford Lake. From the limited sample of 200+ second stadia larvae released into the author's garden pond on 12th and 13th of April none were observed emerging as adults.

The external morphology of eggs of Odonata is well studied (Corbet, 1999) including ultrastructural studies using transmission electron microscopy (Sahlén 1994, 1995). Work by Pierre (1904) over 115 years ago, showed that eggs hatched without being wetted, i.e. into the air surrounding the oviposition scar. The mechanism appears to be well adapted to facilitate the transition of prolarvae of *C. viridis* from an air-only environment to water, where the larvae complete their development. The eggs of *C. viridis* hatch with the prolarvae retaining a thin liquid covering of amniotic fluid, which facilitates leaving the oviposition scar. The surface tension of the liquid is sufficient to maintain contact of the body to the twig by just a few segments, as seen in the video sequence (Plate 4 and Cham 2021b for video link). However, the liquid components of the egg and prolarva are less well understood.

To allow newly emerged 1st stadia prolarvae to get clear of the branch from which they have hatched a leaping movement is employed. This mechanism is rapid, following the twisting of the anterior part of the body. Pierre (1904) states (translated from French) "Usually, at the time of hatching, the rounded half of the egg is slightly protruding. The chorion opens to this level, and the prolarva first straightens itself, that is to say, given the position of the egg, tangentially to the twig". He then observed that, when landing on the ground, prolarvae perform jumps (leaps) of up to 3 cm, with no apparent orientation, for several hours if necessary, which most often end up allowing them to reach permanent water. Here they develop into 2nd stadia larvae. Improvement and better resolving power of modern microscope optics compared to Pierre's time allow better clarity on the nature of the droplets which facilitate egg hatching and ecdysis and would explain the difference in the interpretation described here. The droplets have a range of sizes yet appear to have an ordered and regular repeating pattern. The hydrophobic nature of these droplets on the sheath serves a key role in maintaining the prolarva on the surface during ecdysis, facilitating the 2nd stadia larva to break free on the water surface. Further study is required to establish the origin and chemical properties of these droplets.

The observations in this study indicate the prolarvae do not drop into the water solely by gravity from where they hatch but actively propel themselves away from the plant. It has been stated that prolarvae can travel up to 6 cm after landing (Pierre, 1904) and the distances of around 5 cm observed here concur with that. The prolarvae, which are covered in a thin film of amniotic fluid, are better able to move on hard surfaces than in water. This would serve as a mechanism to prevent premature ecdysis in rain droplets (Corbet, 1999). Whilst at the risk of desiccation, prolarvae have been observed surviving for several hours out of water (Pierre, 1904) and the thin liquid covering may give them some protection for a limited period.

The process and timing of egg hatching may be influenced by a number of factors, including night and daytime air temperatures, aspect, type of plant, plant age and growth rate, proximity of other shading trees, damage to the tree preventing continued growth and potential exposure to parasitoids (Harabis *et al.,* 2019).

At the time of egg hatching, the branches of *Salix* have undergone many changes. The insertion point and scar formed at the point of oviposition are subjected to the vagaries of the environmental conditions thereafter. During the late autumn growth of *Salix*, winter temperatures and spring growth, the size and dimensions of the woody plant tissue in proximity to the oviposition scar will change. The oviposition scar openings and surrounding plant tissue are also subjected to algal and lichen growth during this time, with some openings appearing blocked as a result. Woody branches experience expansion and contraction with varying temperatures, with possible desiccation of the outer woody layers. Combined with the activities of other organisms, such as commensal mites and, potentially, parasitoids (Harabis *et al*., 2019), the eggs are developing in a relatively hostile environment. This supports Pierre (1904), who considered the impenetrability of dry bark surrounding the eggs at the time of hatching to be an impairment for hatching. It is unlikely that hatching 1st stadia prolarvae are able to open up, or modify, openings with woody growth. Martens (1997) reported the potential threat to emerging prolarvae from the resin of conifer branches, which bear oviposition scars.

It has been suggested that twigs where oviposition scars show visible holes is indicative of successful hatching (Tyrrell, 2019). However, the observations in the present study do not support this. Oviposition scars on the branch samples examined closely, showed a combination of no openings, openings of varying sizes or just a slot. The dissection of oviposition scars under the microscope revealed shrivelled eggs, which could easily be misinterpreted as eggs that had successfully hatched. Indeed, at high magnification (64x) the dark pigmented eyespots of perished prolarvae could be seen. The reason for the demise of these is unknown, but in some cases could be explained by the exit opening becoming blocked, preventing development and hatching. In other cases the possibility remains that woody tissue continued to grow around the egg scars, effectively crushing the eggs and thus preventing their development. Caution is, therefore, needed when visually inspecting oviposition scars in the field for signs of successful egg hatching, with observations based on opening shape and size liable to possible misinterpretation.

It has been suggested that prolarvae of *C .viridis* have the ability to emerge from eggs on twigs submerged during periods of flood (Tyrrell 2019). The work of Pierre (1904) and the findings of this study show hatching of eggs and prolarvae of *C. viridis* needs to take place in air and it is difficult to see how the hatching mechanism and prolarval ecdysis could operate in conditions underwater. To be able to extract itself from the prolarval sheath and prevent premature hatching, the prolarva needs to initially float on a horizontal water surface, which is promoted by the strongly hydrophobic droplets associated with the sheath. In this study, all branch samples with unhatched eggs failed to hatch during or after periods of submergence and subsequent removal from water. Duffy (1994) estimated mortality in the egg stage of *Lestes disjunctus*, which oviposits above the water line, to be around 22.6% of which 16.6% was due to loss resulting from flooding events, and Corbet (1999) considered this to be an underestimate. Further research is required to establish if there is a possible mechanism for hatching when submerged.

Conclusions

The initial objective of this study was to establish the timing of actual, rather than inferred, egg hatching of *Chalcolestes viridis* for the first time since its colonisation in Britain. From the limited sample investigated, eggs hatched throughout April 2020, with individual branches exhibiting a hatching period of 7-8 days. Egg hatching and larval development under fully natural conditions is difficult to observe and assess. The results presented here fit with observations across Europe that eggs hatch during April, followed by rapid larval development, with adults emerging during July. Care needs to be taken when visually assessing oviposition scars and their openings as an indicator of successful hatching, particularly when branches have been exposed to exceptional events such as flooding.

The process of egg hatching and emergence of 2nd stadia larvae from 1st stadia prolarvae concur with those described by Pierre (1904). The prolarva is well adapted to extricate itself from the oviposition scar and leap clear from the twig where it hatched. If the prolarva lands on dry ground, the same leaping mechanism is used to enable it to get to permanent water. On reaching water, the emergence of the 2nd stadia larva requires the prolarva to float on a horizontal water surface to enable complete ecdysis at this stage, and this is facilitated by the hydrophobic properties of the prolarval sheath

Since its colonisation of the UK, *C. viridis* has become very abundant in many areas where it has been recorded. It would be useful to repeat these studies where *C.viridis* has been observed ovipositing in other woody plants, and where the possibility might exist to monitor egg development more closely.

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Video link

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The last populations of *Leucorrhinia dubia* (Vander Linden) (White-faced Darter) at Claife Heights, Windermere, Cumbria?

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Summary

A small native population of *Leucorrhinia dubia* (White-faced Darter) is known to have survived for nearly a century on Claife Heights, Windermere. Its history is outlined, including the recent apparent attempts to colonise alternative sites in the area and its ultimate decline and probable extinction following low population levels. The potential of the area for future use by the species is considered.

Introduction

Claife Heights is a forested upland area some 8 km^2 in extent above the western shore of Windermere, opposite the Cumbria town of that name (Plate 1). (The area fell within the Furness district of Lancashire until 1974). Average altitudes reach some 200 metres a.s.l. The earliest records of *Leucorrhinia dubia* (White-faced Darter) there appear to be those of the Freshwater Biological Association's entomologist T. T. Macan, who bred out a few larvae in the 1930s. Adults (Plate 2) and larvae were collected in 1943 from the same site for the then British Museum, now the Natural History Museum (Kimmins, 1943). The precise location never appeared in print, though a mire near Renny Crags, named Green Tarns (SD365985) on O.S. maps, and usually referred to as 'Green Pool' by Macan, was evidently the only site – as a search of his archive revealed (Clarke, 2019). The continuity of the species at Green Tarns can thus be traced back to about 1935.

The Green Tarns site (again without being named as such) was evidently still occupied in 1953 (Ford, 1953) but seems to have become unsuitable for *L. dubia* by some time in the early 1990s. Stephen Hewitt and the author saw a mating pair and two other adults there in 1989 and, apart from an exuvia found by Mo Richards in 1992, there are no later confirmed records. Like many mire sites, Green Tarns has been subject to successional changes and gradually

Plate 1. Claife Heights: location in Cumbria. Contains OS data © Crown copyright database rights 2021.

Plate 2. One of four adult specimens of *Leucorrhinia dubia* collected for the British Museum in 1943 from Claife Heights. Courtesy of the Trustees of the Natural History Museum.

infilled, losing its open water. Adjacent forestry plantations may have hastened this decline.

Post-1990 developments

It was a relief that on 13 July 1993 Stephen Hewitt, John Cubby of the Forestry Commission, Ian Slater of English Nature and the author, found *Leucorrhinia dubia* at Brown Stone Moss on Claife. The site itself was not previously known to us, and the apparent 'population' was tiny – two exuviae, two single adults and a mating pair.

The site (Plate 3) is a small tarn of about 0.33Ha at SD381976. It is formed by a dam and was probably used as a fire pond. It has clear, deep, moderately acid water, with typical breeding Odonata of Lake District forest tarns, such as *Cordulia aenea* (Downy Emerald) and *Aeshna cyanea* (Southern Hawker).

Plate 3. Brown Stone Moss from the north end, 2012.

There are ‘islands’ formed from material that has lifted off the pool bottom and become encrusted with *Sphagnum*, though this is not *Sphagnum cuspidatum* – which is fully aquatic and the larval habitat of *L. dubia*. Floating *S. cuspidatum* has never been extensive; it has decreased over the years and is now limited to a short stretch of the NW edge. Exposure has increased recently, owing to the felling of mature trees that previously provided shelter.

It is fortunate that others have been able to visit this rather remote site more often than the original finders. From 2008, Colin Adams visited at least once in most years and Mo Richards also made many visits. Heather and Tony Marshall are the only recorders to have covered the emergence period thoroughly, making nine visits in 2002 (27 May – 13 July) and seven in 2003 (25 May – 16 July). These visits were supported by the Forestry Commission as part of their action to support the Cumbria Biodiversity Action Plan (Marshall & Marshall, 2003, 2004). Unsurprisingly, overall exuvia totals were much higher than achieved in single visits, though adults were in no greater abundance than observed on any one day in most years. The exuvia counts in the two years concerned were very similar (Table 1).

Table 1. Summary of the results from the 2002 and 2003 surveys at Brown Stone Moss carried out by Heather and Tony Marshall with Forestry Commission support.

2002			2003		
Date	**Adults**	**Exuviae**	**Date**	**Adults**	**Exuviae**
27 May	None	2	25 May	2 emerging	2
01 June	2 emerging	15	30 May	1 emerging	26
09 June	7 emerging	21	07 June	1 male flying	14
15 June	1 emerging	2	14 June	2 males flying	2
23 June	None	3	22 June	None	3
30 June	None	2	29 June	2 males flying	0
06 July	None	0	-	-	-
13 July	1 male flying	0	-	-	-
20 July	None	0	16 July	None	0
Total emerged in 2002		45	Total emerged in 2003		45

No counts of flying adults present at Brown Stone Moss on single day visits by any observer have ever attained double figures. Numbers since 2010 have been particularly low, the highest being one male, two females and a mating pair noted on 11 June 2010 by Colin Adams. In 2013, he found three tenerals on 8 June and three matures on 6 July (which could have been the individuals seen earlier). Since then, numbers of adults or exuviae have never exceeded two on any one occasion and none were seen in 2016, 2017 or 2019. On 1 June 2020, Mo Richards found a single exuvia (Plate 4) – indicating that oviposition probably occurred in either 2017 or 2018.

It may be more than a coincidence that another previously unknown population of *L. dubia* was found at Tongue Intake Plantation (NY327026), about 6km NW of Green Tarns, in 1999 by Heather Marshall (Marshall & Clarke, 2000). The pool there is of similar extent to Brown Stone Moss, though enclosed by deciduous woodland and receives some enrichment from leaf-fall. There is a dam at the outflow. *Sphagnum cuspidatum* is confined to a short length of the western margin of the tarn (Plate 5). This marginal raft echoes the final situation at Brown Stone Moss, where the only area *L. dubia* was using was also a marginal raft. A count of about 80 exuviae was made on 1 July 2000 by the author and others. The annual number of exuviae soon dwindled and the colony had vanished by about 2010. While the presence of fish could be a factor in the decline, none were noted during a survey for *Hirudo medicinalis* (Medicinal Leech) in 1999 (H. Marshall, pers. comm.).

Plate 4. *Leucorrhinia dubia* exuvia at Brown Stone Moss, Claife Heights, 2020. Photograph by Mo Richards.

Discussion

Before the Marshalls' data at Brown Stone Moss became available (Marshall & Marshall, 2003, 2004), it was felt that the very low numbers of *Leucorrhinia dubia* suggested that the colony's continuity there might have depended on the existence of populations elsewhere on Claife. This now seems unnecessary – and unlikely. Nonetheless, the persistence of the species at Brown Stone Moss in such low numbers for the last decade seems remarkable. Changes at the site, with reduction in *Sphagnum cuspidatum* rafts, may have caused a decline. It is possible that inbreeding could also have contributed.

It is tempting to suggest that both the Brown Stone Moss and Tongue Intake colonies represent attempts by the species to find alternative habitats in the face of the decline of the Green Tarns site. They are about 2km and 6km respectively from that site. Both may perhaps be considered 'second class' in terms of their suitability for the species – neither is a classic peat bog.

Plate 5. The tarn at Tongue Intake Plantation, 2007. The small area of marginal *Sphagnum* is arrowed.

The species is especially noted for not usually moving far from breeding sites. Mo Richards' finding of two exuviae at Highs Moss (SD375980) (Plate 6) in June 2006 may have been the outcome of a very local dispersal to that site, which is less than 1 km from Brown Stone Moss. Searches in recent years by Mo Richards, Richard Tanner, the author and others for further colonies have all yielded negative results. The mystery thus remains: it is still impossible to be completely sure whether the species is extinct at Brown Stone Moss, or whether it occurs elsewhere in the wider Claife area, which is dotted with mires and other wetlands amidst modern forestry.

Despite their apparent visual appeal, the larger named Mosses (mires) on Claife – Highs Moss and Nor Moss – appear to have water that is too mineralised to sustain significant *S. cuspidatum* rafts and thus *L. dubia*. The greater wind-exposure of their water bodies may also be a factor. It is possible, as Macan's experience seems to bear out, that *L. dubia* has never been widespread on Claife since records began. The hydrology of the area is extremely complex: the predominant mire type is valley mire, with some water flow, rather than static basin mires and peat-bogs that *L. dubia* tends to favour. Small-scale domestic

Plate 6. Highs Moss, Claife Heights, 2012.

peat extraction may have emulated the latter type very locally, as could even be the case at Green Tarns. Nonetheless, the potential for habitat creation/ restoration within this extensive area clearly exists and the general environment has many echoes of the forest-bogs that some *Leucorrhinia* species occupy in more northern parts of their range in Britain and Europe.

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