# Sampling implications of variation in daily activity of the sheep tick, Ixodes ricinus at a coastal grassland site in the UK

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# **Sampling implications of variation in daily activity of *Ixodes ricinus* sheep ticks at a coastal grassland site in the UK**

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## Running Title

Tick daily activity affects collection

## Abstract

The sheep tick, *Ixodes ricinus* L. (Acari: Ixodidae) is an important vector of many pathogens of medical and veterinary significance. Determining vector abundance is a requisite of assessing potential vector-borne disease risk. Estimation of tick abundance is often conducted by blanket drag sampling a site, conducted at one time point during the day. The time of day chosen for sampling can vary, is not widely standardised and is often un-reported by the investigator. This study investigated whether the time of day chosen for sampling had an effect on tick collection at an open grassland coastal site in North Devon, UK during May to July 2019. Tick abundance for both adults and nymphs in the evening period was more than twice that found in the mid-day sampling period. Overall abundance differed with site aspect, ground temperature and relative humidity. This study shows that for this open grassland recreational site, the time of day chosen for sampling has important implications for tick collection and the assessment of the relative risk of human exposure to ticks and tick-borne infections.

Keywords: aspect, blanket dragging, bracken, open grassland, tick collection, tick hazard.

## Introduction

The most abundant of the UK tick species is *Ixodes ricinus* L. (Acari: Ixodidae) accounting for 60% of species found in the 2010-2016 Public Health England surveillance scheme (Medlock *et al.,* 2018). It is widespread but patchily distributed, being found widely in woodland and rough grassland habitats with an expanding distribution particularly in the south of England (Cull *et al.,* 2018; Medlock *et al.,* 2018).

*Ixodes ricinus*, commonly known as the sheep tick is the most important vector of pathogens in the UK and its impacts on livestock and human health are of increasing concern (Baines *et al*. 2019). Bovine babesiosis in cattle caused by the piroplasm *Babesia divergens* and vectored by the adult *I. ricinus*, causes significant morbidity and mortality, of sporadic incidence but increasingly reported in conservation grazing. The transmission of louping ill virus, a *Flavivirus*, by *I. ricinus* causes major economic losses in both upland sheep farming and moorland shoots of red grouse (Baines *et al.* 2019). Lyme borreliosis is the most prevalent tick-borne zoonotic infection across Western Europe. It is caused by *Borrelia burgdorferi* sensu lato complex and is transmitted by *I. ricinus* (Tulloch *et al.* 2019). Increasing surveillance by public health organisations and greater public awareness have resulted in the incidence of infections increasing in many European countries, with confirmed cases rising over four fold in the UK within the last decade (Medlock *et al.* 2008, 2018; Tulloch *et al.* 2019).

*Ixodes ricinus* is a mainly free-living generalist ectoparasite, the survival for each life-stage depending on the tick adopting an ambush strategy known as questing, that involves ascending to the vegetation tip; waiting for a passing host to brush past and then attach to obtain a single blood meal. Once engorged, ticks drop from the host and descend into the litter mat. Questing ticks are vulnerable to environmental conditions particularly desiccation threats, mitigation requiring descent to the more humid litter mat to rehydrate (Randolph and Storey, 1999). The daily activity rhythm of cycles of ascent and descent within the vegetation are a direct response to environmental factors, particularly temperature, relative humidity (RH) and photoperiod, involving endogenous rhythms and host activity acting in concert (Lane *et al.,* 1995; Mejlon, 1997). This behaviour can be exploited for sampling by collecting questing ticks that have ascended vegetation. This method uses blanket dragging to produce an estimate of tick density and is one way of assessing the risk of human activity encountering ticks and tick-borne pathogens (Milne, 1943).

In contrast to the investigation of seasonal phenology, few studies have investigated the daily activity patterns of ixodid ticks (Schulze *et al.,* 2001; Madden and Madden, 2005; Dubie *et al.,* 2018). For *I. ricinus* there have been contrasting results for the different life-stages and habitat types. Research conducted on daily activity has found a predominately diurnal pattern on hill pasture for adults and nymphs (Lees and Milne, 1951); mainly nocturnal activity for larvae and adults in meadows but with no discernible pattern in a nearby forest (Mejlon, 1997); and nocturnal activity for nymphs in an open woodland (van Gent, 2009).

If *I. ricinus* daily activity is not accounted for in daytime sampling surveys there is the potential for tick abundance estimates to be underestimated particularly for tick species with predominately nocturnal populations (Mejlon, 1997; Madden and Madden, 2005). Despite the compelling evidence of variation in *I. ricinus* daily activity and its potential to affect tick collection and the determination of relative abundance, the time of sampling is infrequently reported in studies. When reported, sampling is more often conducted for a short time point at any time during daylight hours, times vary and are not standardised.

This study investigated if the time of day of sampling affected the collection of questing ticks, and related this to environmental factors including aspect at a coastal grassland conservation area in North Devon, UK, during the spring and early summer period. This area is crossed by many walking routes and subject to high human visitor pressure and importantly this study may have a bearing on the assessment of tick hazard for humans visiting this recreational site.

## Materials and Methods

### *Study Area*

The study was undertaken at Bull Point (51.200 N, 4.220 W), near Woolacombe, North Devon, UK, comprising a coastal conservation and recreational area of about 23ha. Bull Point (90m a.s.l.) is at the end of a northward directed ridge having rugged cliffs backed by steep slopes to the east and south west descending to sheltered rough pastureland. The area has underlying geology of slates and poorly drained soils. Vegetation is a mosaic of coastal heathland, acid/neutral grassland, treeless with scrub communities restricted to the well demarcated rocky outcrops that dissect the grassy slopes. Bracken (*Pteridium aquilinum*) encroachment of the lower slopes is profound and centred around the scrubby outcrops; accessible areas of the grassland and bracken were mown every September. There is limited sheep grazing in the spring; the ewes and lambs introduced on May 14 were moved off on June 10 when there was insufficient grazing; only the lambs were treated with a prophylactic acaricide. Roe deer (*Capreolus capreolus*) range throughout the area and there is evidence of small mammals and ground feeding birds. The area is exposed to prevailing south westerly winds leaving it windswept and with precipitation ranging from 900-1000mm per annum.

*Study sites and tick collection*

An initial survey conducted over three consecutive days in early May 2019 compared sites on three different grassland slopes of south east (SE), north west (NW) and south (S) aspects, consisting of mixed grasses (10-15cm height) and scattered emerging bracken croziers. The study plots orientated along the slope were 100m X 30m and subdivided into 10m X 30m sub-plots. Of these ten sub-plots, eight were randomly assigned (by drawing lots) to one hour sampling periods that took place between 09:30 hrs to 20:30 hrs BST. Sub-plots were adjacent to each other as ticks in-field have a very limited movement horizontally, favouring vertical movement (Lees and Milne, 1951).

For each sub-plot 15 parallel 10-$m^2$ drags were carried out in the one hour sampling period, walking at a slow pace with a 1-$m^2$ white loop-stitched cotton towelling material held square, that included a metal rod in the hem of the trailing edge to maintain contact with the vegetation. At the end of the drag both sides of the blanket were examined, ticks removed, recorded by sex, life-stage and released; up to three ticks (adults and/or nymphs) were retained from each one hour sampling period and were stored in 70% alcohol, with a total of 33 adults and 61 nymphs examined for species confirmation (Arthur, 1963). Larvae were excluded from analysis because of the difficulty of quick but reliable identification in the field. Ground temperature and RH at 5cm above the soil surface was recorded with a hand-held hygro-thermometer (UT333 mini meter, Unit-T, China; RH±5%, temperature±1.0 °C) at the end of each drag.

General weather conditions and vegetation type and height were noted for each plot. Wind speed and direction, cloud cover and overnight temperatures were not recorded. Sampling was not carried out when the vegetation was wet with dew or after rainfall.

After the initial survey in early May, the main study was centred on the SE-facing slopes because of the greater tick abundance and suitability for locating multiple plots. By mid-June, the dominance of bracken patches and clumps of tall grasses (up to 60cm height) on the lower SE-facing slopes had complicated dragging; reducing collection efficiency and making it necessary for the later survey plots on June 17 and 20 and July 8 and 10 to be moved to the uppermost areas of the SE-facing slopes. This was a large open area of short mixed species grassland (5-15cm height) with a few isolated bracken patches peripherally; there was indication of preferential sheep grazing and a public footpath running along one side. By mid-July there had only been moderate grass growth (10-20cm height). The area was sufficient for two adjacent study plots to be marked out, avoiding the bracken patches; one plot was surveyed on June 17 and July 8 and the other on June 20 and July 10.

*Statistical Analysis*

The ticks (adults + nymphs) collected by the fifteen $10m^2$ drags in each one hour sampling period, were used to calculate a mean of adults and nymphs collected for each $10m^2$ drag. Results obtained for each of the eight individual hour long time periods sampled were combined to produce four, two hour time periods (1=09:30-11:30hrs, 2=12:30-14:30hrs, 3=15:30-17:30hrs, 4=18:30-20:30hrs) to increase statistical reliability. The mean number of ticks/drag provided an estimate of density, the relative index of abundance (RA) was calculated for individual two-hour sampling periods (30 drags) and for the whole day sampling period (120 drags) (Table 1). Collection data was over-dispersed and non-normally distributed. The means of total tick and nymph numbers were compared using Kruskal-Wallis rank sum chi-squared test with Wilcoxon rank sum pairwise comparison and Bonferroni *p*-value adjustment to compare between time periods. For both temperature and RH, regression with

Poisson errors revealed overdispersion as the residual deviance was much greater than its associated degrees of freedom, so a new model with quasipoisson was fitted. Data on tick density and environmental measurements were analysed using R statistical software (R version 3.5.1., 2018).

## Results

The initial survey in early May of the SE, NW and S slopes collected 150 (5 adults, 145 nymphs), 52 (5 adults, 47 nymphs) and 18 (3 adults, 15 nymphs) ticks on their respective slopes.

For the main study of the SE-facing slopes, six hundred 10m$^2$ drags collected 853 *I. ricinus* ticks, 114 adults (56 males and 58 females) and 739 nymphs (Table 1). The ratio of adults to nymphs was 13.3% and 86.7%, respectively. The overall ratio of adult males to females was 1.0:1.03.

Sampling on May 12, was conducted on the lower parts of the SE-facing slopes, further sampling in June and July took place on the upper parts of the SE-facing slopes (Table 1). Morning and evening exhibited low temperatures (mean 16.1°C) and high humidity (mean 73.3% RH) with the middle of the day showing the highest temperatures (mean 22.1°C) and lowest humidity (mean 63.5% RH). Tick abundance decreased with increasing temperature (t=-4.34, d.f.=38, $p$<0.001) and abundance increased with increasing humidity (t=2.08, d.f.=38, $p$<0.05).

The total ticks (adult+nymphs) collected during the middle and warmest period of the day (time period 2, 12:30-14:30 hrs) varied with the date of sampling: May 12, total ticks 17 with mean temp (±SEM) 17.2 (±0.22)°C; June 17, total ticks 33 with mean temp 17.2 (±0.16)°C; June 20, total ticks 40 with mean temp 20.2 (±0.22)°C; July 8, total ticks 24 with mean temp 24.5 (±0.28)°C; July 10, total ticks 8 with mean temp 26.6 (±0.21)°C. From June 17 to July 10 as the middle of the day temperature increased from 17.2°C to 26.6°C, the number of ticks collected decreased from 33 to 8 (t=-1.73, d.f=3, $p$=0.05).

As sampling continued June to July, the mean RA/10m$^2$drags/day decreased with concomitant decrease in % positive drags (drags that collected at least one tick) and range/drag (greatest number of ticks collected in an individual drag) (Table 1). Abundance differed when comparing time periods across the day, generally decreasing from period 1 (09:30-11:30) reaching the lowest value at period 2 (12:30-14:30) then increasing to greatest abundance in period 4 (18:30-20:30).

## Discussion

This small study, the first tick survey of Bull Point as far as is known, focussed on the SE-facing grassland slopes, finding that both adults and nymphs exhibited a significant daily activity pattern when comparing sampling sessions completed during the survey period. Overall RA for total ticks and nymphs was lowest in the middle period of the day (time period 2, 12:30-14:30hrs), in the afternoon (time period 3, 15:30-17:30hrs) it had increased by a half and by evening (time period 4, 18:30-20:30hrs) RA had more than doubled compared to the middle period of the day (Figure 1 and Table 1). Of the few field studies on *I. ricinus* daily activity, only two have specifically investigated open habitats of meadow and grassland; Mejlon (1997) compared activity of forest and open meadows in Sweden, finding in the meadow that activity was minimal in the middle of the day but increased to a peak at 23:00-03:00 hrs, however in the forest there were no differences in tick activity throughout the day. Field experiments by Lees and Milne (1951) on Northumberland hill pastures in northern England concluded that I. ricinus activity was mainly diurnal, moderately increasing in the afternoon and decreasing at night. The results of this investigation shows a significant increase in the afternoon and evening activity that agrees with both authors and is believed to be the first report demonstrating daily activity pattern of adult and nymph *I. ricinus* ticks in a field survey of an open grassland coastal site in the UK. The coastal peninsula of Gower, south Wales, UK, is a diverse area of grassland, heath and forest; an extensive study by Medlock *et al.* (2008) investigated ecological and environmental variables that could be used to predict presence and abundance of *I. ricinus.* Finding the most optimal predictor variables that included; calcareous and neutral grassland, dwarf heath, damp impermeable soils, the presence of grazing cattle and sheep, a lower midday temperature and topographical features of aspect and a reduced slope. Many of these predictor variables were also present at the current study of Bull Point.

The decision to concentrate the survey on the SE-facing slope was influenced by the findings from the initial survey in early May of the large differences in RA/day between aspects, RA being greatest on the SE and least on NW and S-facing slopes. This appears to agree with Medlock *et al.* (2008) findings of W, SW and SE being optimal aspects for *I. ricinus* abundance compared to the suboptimal aspects of S, NE and NW.

In the British Isles *I. ricinus* is associated with a variety of habitats, particularly areas containing vegetation that maintains an adequately moisture rich environment in the litter layer that mitigates desiccation (Walker *et al.,* 2001; Tack *et al.,* 2012). Abundance of ticks is generally higher in woodland and scrub as the vegetation provides a cooler, more humid stable microclimate as well as forage and shelter for host species; with abundance being lower in open biotopes such as grassland. This study concentrated on sampling the more exposed open grassland avoiding the more favourable tick habitats of bracken patches and scrub. The moderate tick abundance found in this grassland area can be attributed to the high precipitation, a dense sward and humid litter mat providing a tick favourable microenvironment along with the sheep and wild hosts that effect tick distribution from the bracken and scrubby areas (Sheaves and Brown, 1995). Despite having a short grazing period (May 14 to June 10), the high density of ewes and lambs would have mopped up many questing tick of all life-stages affecting the numbers collected; those feeding on the lambs would have perished due to their previous treatment with acaricide, with the ewes widely distributing engorged ticks throughout the area. The sites in June and July were sampled twice and it could be argued, that because collected ticks were released after identification then a small proportion of ticks collected in July could have been those previously collected in June; the influence of this on the overall results was considered insignificant.

The importance of a number of environmental factors relating to tick abundance and activity was demonstrated in this survey. This was apparent in the changes observed in RA that followed the diurnal differences in ground temperature and RH that were not unexpected in view of the tick's optimal environmental requirements for survival and activity, agreeing with the observations made by Mejlon (1997) and Medlock *et al.* (2008). The fall in light intensity and its influence on RH and ground temperature, along with changes in host activity could account for the increasing tick activity observed in the evening (Greenfield, 2011; van Gent, 2009; Zöldi *et al.,* 2013). Tick activity in the middle of the day (time period 2, 12:30-14:30hrs) varied during the study. The overall decrease in tick numbers collected as the temperature increased from June 17 to July 10 could be associated with reduced tick activity mitigating desiccation; additional factors such as wind speed, RH and the normal seasonal change underlying activity particularly the timing of the spring peak during the study period will affect tick collection (Medlock *et al.* 2008). When comparing May 12 (17) and June 17 (33) both having identical midday mean temperatures, the number of ticks collected on May 12 would have been expected to be greater than those collected on June 17, particularly as seasonal peak abundance would have encompassed May 12 and abundance would have been decreasing by June 17 (Randolph *et al.* 2002). The reduced number of ticks collected on May 12 could have been influenced by the low overnight temperature on May 11-12 (5°C, author's observation) and the low RH affecting tick activity. The greater abundance recorded for the whole day and midday period on June 17 compared to June 20 (Table 1) can be attributed to the cloudy overcast sky reducing insolation, influencing ground RH and temperature; the result being an improvement in tick microclimate more favourable for questing (Greenfield 2011; Medlock *et al.,* 2008). The decrease in RA from June 17 to July 10 (Table 1) affecting all time periods is explained by the normal seasonal decline that occurs after the period of peak abundance probably in late April to early May (Randolph *et al.* 2002). Questing tick numbers decrease because the rate of attachment or death as the season progresses exceeds recruitment of unfed ticks from the litter mat (Randolph *et al.* 2002).

Daily activity of *I. ricinus* is poorly recognised in open habitats, this study demonstrated that the time of day chosen for sampling has a significant influence on tick collection on this particular open grassland site with questing activity in the evening period being over twice that of the mid-day period. Surveys that investigate the risk of human tick exposure, by necessity sample sites during the period of the day concomitant with high visitor numbers. It could be argued that surveying in the evening and night time are rarely necessary because there are fewer visitors, but sampling times should be considered on a case by case basis particularly for popular recreational areas. For instance, camping areas and festival sites are a special case and should be surveyed preferably in the evening to assess risk. The author observed many evening visitors to Bull Point socialising and viewing the sunset, importantly coinciding with the period of increased tick activity.

Some visitors were aware of ticks and tick-borne diseases, and believed that the patches of bracken and scrubby areas potentially harboured many ticks, were more hazardous and should be avoided; whereas the open grassland was considered 'safer' to be used for picnicking and resting. The significant tick activity within this grassland and risk for human exposure to tick-borne disease should be emphasized to visitors.

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Table 1. Summary of the number of ticks collected, mean (±SEM) relative abundance (RA)/$10m^2$ drag for total ticks (adults+nymphs) including weather observations for the SE-facing slopes sampled between May to July.

| Date | Total ticks (Tt) | Adults(♂/♀) Nymphs | RA day 09:30-20:30 | RA 09:30-11:30 | RA 12:30-14:30 | RA 15:30-17:30 | RA 18:30-20:30 | % positive drags (Range Tt/drag) | Weather Temp °C RH % (means) |
|---|---|---|---|---|---|---|---|---|---|
| **May 12th** | 150 | 1/4<br>145 | 1.25<br>±0.11 | 1.13<br>±0.2 | 0.56<br>±0.15 | 1.46<br>±.0.21 | 1.83<br>±0.24 | 65%<br>(0-5) | Sunny<br>13-19 (16)°C<br>54-86 (66)% |
| **June 17th** | 326 | 18/22<br>286 | 2.72<br>±0.27 | 1.06<br>±0.25 | 1.1<br>±0.26 | 3.3<br>±0.42 | 5.36<br>±0.68 | 76%<br>(0-17) | Overcast<br>15-19 (16)°C<br>69-88 (76)% |
| **June 20th** | 204 | 23/15<br>166 | 1.7<br>±0.13 | 1.16<br>±0.22 | 1.33<br>±0.19 | 1.76<br>±0.28 | 2.53<br>±0.29 | 80%<br>(0-6) | Sunny<br>14-23 (20)°C<br>56-80 (67)% |
| **July 8th** | 109 | 6/11<br>92 | 0.91<br>±0.09 | 0.96<br>±0.17 | 0.8<br>±0.14 | 0.96<br>±0.18 | 0.9<br>±0.24 | 59%<br>(0-4) | Sunny<br>17-30 (23)°C<br>51-81 (65)% |
| **July 10th** | 64 | 8/6<br>50 | 0.53<br>±0.06 | 0.5<br>±0.13 | 0.26<br>±0.08 | 0.46<br>±0.12 | 0.9<br>±0.14 | 41%<br>(0-3) | Sunny<br>17-24 (23)°C<br>52-87 (71)% |

Note :Relative abundance (RA) day mean±SEM ticks/$10m^2$ calculations based on ticks collected in 120 drags and for individual time period RA mean±SEM ticks/$10m^2$ calculations based on ticks collected in 30 drags. %positive drags=drags finding at least one tick. Range Tt/drag=most ticks collected in an individual drag.

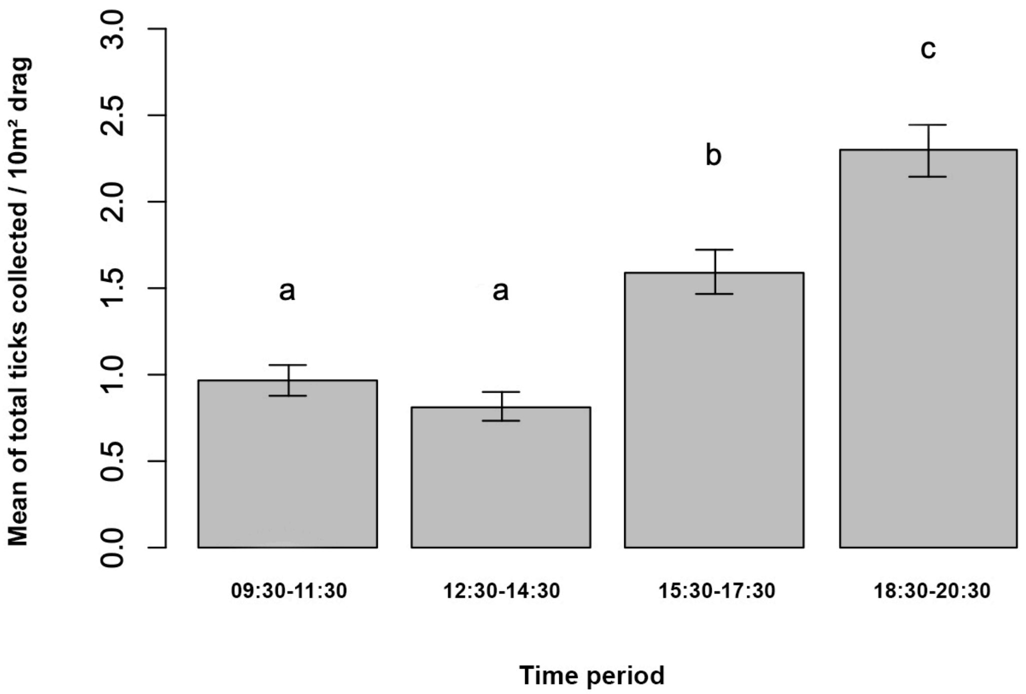


Figure 1A. Relative Abundance (RA) expressed as mean of total ticks (adults+nymphs) collected/10m$^2$ drag sampled between May to July for each combined time period. All times BST. Kruskal-Wallis rank sum test chi-squared=55.22, d.f.=3, *p* values=6.16e-12. a-a, *p*=1.00, a-b *p*=0.00012, a-c *p*<0.0001, b-c *p*=0.0083.

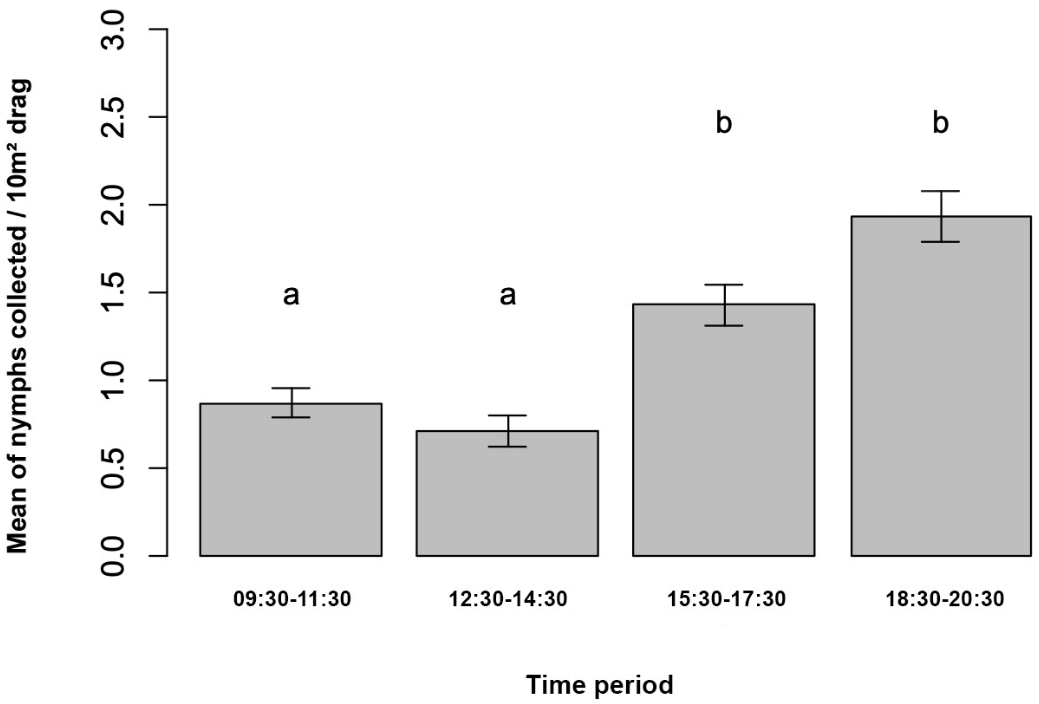


Figure IB. RA expressed as mean of nymphs collected/10m$^2$ drag sampled between May to July for each combined time period. All times BST. Kruskal-Wallis chi-squared=50.09, d.f.=3, *p*-value=7.62e-11; a-a *p*=0.625, a-b *p*<0.0001, b-b *p*=0.070.