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Rare Events in Remote Dark Field Spectroscopy: An Ecological Case study of Insects

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***Abstract*—A novel detection scheme for the monitoring of insect ecosystems is presented. Our method is based on the remote acquisition of passive sunlight scattering by two insect species. Procedures to identify rare events in remote dark field spectroscopy are explained. We further demonstrate how to reduce the spectral representation, and how to discriminate between sexes, using a hierarchical clustering analysis. One-day cycle showing the temporal activities of the two sexes, as well as data on activity patterns in relation to temperature and wind are presented. We also give a few examples of the potential use of the technique for studying interactions between sexes on a time scale of milliseconds.**

Remote sensing, Ecosystems, Entomology, Insects, Calopteryx, Passive Scattering Spectroscopy, Dark Field Spectroscopy.

I. Introduction

THE application of modern spectroscopic methods to monitor several larger constituents of the atmosphere, such as birds and insects, has previously been demonstrated by our group. In earlier papers we mainly focused on the use of LIght Detection And Ranging (LIDAR) [1]-[5], and presented several approaches to insect marking experiments using Laser Induced Fluorescence (LIF). We are currently exploring several more compact and less expensive approaches. Here, we present dark field spectroscopy, a monitoring method that does not require the marking of organisms. This enables the study of species that can not be easily handled, or are difficult to capture. In addition, natural densities of organisms can be more accurately estimated with the current approach, because no population subsets need to be measured, but instead, whole population samples within designate areas can be investigated. Traditionally, manual counts have been used to quantify the numbers of individuals of different species of damselflies and for studying the temporal activity patterns and interactions between species. However, manual observations are time consuming, and simultaneous monitoring of long river stretches over long time periods is laborious. In addition, interactions between damselflies on short timescales are easily missed during manual counts. The presented inexpensive method may therefore enable biologists to address new questions regarding temporal activity patterns in relation to weather conditions, like in this study, or other ecological variables.

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II. Background

A. Model Organism

The river Klingavälsån in southern Sweden is occupied by two closely related, ecologically similar [6] and co-existing populations of damselfly species: *Calopteryx splendens* and *C. virgo.* For this reason, this location provides an excellent place to test new methods that can detect and identify both the different species as well as the sexes of these damselflies by making use of their slightly different scattering spectra. Damselflies are excellent biomarkers, since they have both an aquatic larval phase and a terrestrial adult phase, and thus can be used as indicators both of water and terrestrial habitat quality. Calopterygid damselflies are particularly suited as biomarkers because they inhabit flowing rivers with clean water and are sensitive to low oxygen conditions [6], [7]. In addition to being suitable biomarkers for habitat quality, damselflies are also sensitive to temperature changes [8], and *C. splendens* and *C. virgo* are predicted to shift their ranges northwards in response to the global warming [9]. Simple quantitative monitoring of damselflies can therefore be useful both for investigations of freshwater and terrestrial ecosystem quality, as well as for the study of the effects of changing climatic conditions. In this study we monitor flying damselflies in their adult, terrestrial phase, which lasts for a few weeks during the summer months.

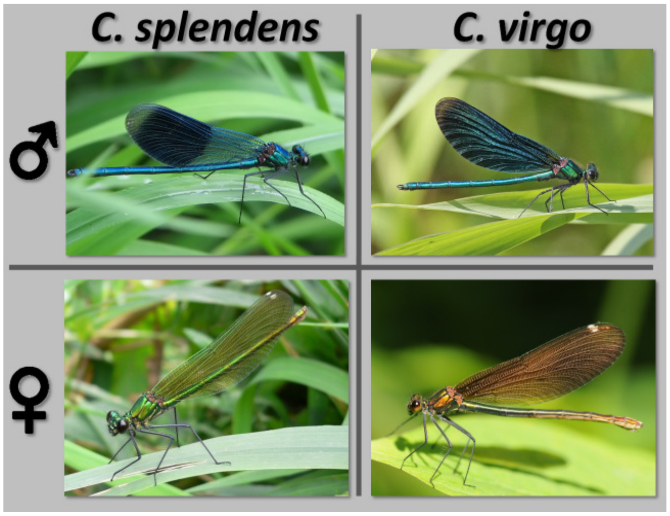

Fig. 1. Study organisms. To the left, a male and a female of the banded damselfly *Calopteryx splendens* are shown, and to the right a male and a female of the blue damselfly *Calopteryx virgo*.

B. Spectral Appearance

Calopteryx spp. are diurnal and have striking blue and green-brown colored bodies. These colors are created by a combination of structural metallic colors arising from coherent scattering from prevalent spatial frequencies in a nano-arrangement of organelles and chromophores [10]; see Fig 1. Although the phenomenon is structural, the spectral features show no iridescence due to the spherical symmetry of the nano-arrays [10]. Females of *C. splendens* are greenish, whereas *C. virgo* females are more brownish. Females of both species have wing colors similar to their body; see Fig. 1; [3]. In contrast, males of both species have a blue body, and conspicuously melanised wings. *C. virgo* males have melanised wing-spots covering most of the wing (~90%), whereas *C. splendens* males have wing-spots, which cover around half of the wing; see Fig. 1; [3]. The differences in coloration between the sexes and species could be the result of sexual selection, and since males have higher variance in reproductive success, they are expected to be under stronger sexual selection [11]. The conspicuous dark body and wing color of males can be more easily spotted than the color of females (against the vegetation background), and the melanised wing patches of the males have also proven important for species recognition in co-existing populations [12]. Newly emerged and not fully hardened specimens [6] show increased specular reflectance, and can be distinguished by their glittering appearance from more mature individuals that have a hardened exoskeleton [13]. The habitat area studied in the present study is the air volume above the river surface, and this area is generally not utilized by newly emerged individuals but typically exclusively by adult sexually mature damselfly individuals.

C. Visual System

Several dragonfly species have been found to have up to six spectral classes of photoreceptors (spectral bands) covering the range 330-650 nm [14]. Although no such studies have been done on damselflies so far, dragonflies and damselflies are sister taxa and hence likely to have similar visual systems. Damselflies have compound eyes and have a rather poor spatial resolution compared to humans, but an extremely wide field of view, and a fast temporal response. Another interesting aspect of the damselfly visual system is the ability to detect polarized light, a feature which is common in insects, and in particular in species living over the water surface. The ability to detect polarized light has several implications for both the perception of structural colors and for horizon estimation during flight navigation.

III. Material and Methods

A. A River System with two Co-existing Damselfly Species

The river site in the Klingavälsån nature reserve (Lat: 55.63°, Long: 13.54°) harbours a population consisting of both *C. splendens* and *C. virgo* damselflies. These damselfly populations have been extensively studied as model organisms for sexual selection, species recognition and predation [15]-[17]. Studies of damselfly distribution and activity patterns have also been performed with fluorescence LIDAR in this population [4].

In order to compare the developed method to traditionally employed methods, manual counts of damselflies along the same stretch as used in the dark field spectroscopy study were done. These counts were performed every 30 minutes. Species and sex of all damselflies above the river surface was estimated and recorded. A synchronized digital still camera was used to take pictures for cross validation, and additional observations were recorded manually in a log book.

B. Equipment and Optical Setup

The one-day experiment was carried out using instruments placed in an astronomical tent-dome observatory (Omegon, approx. ø 3 m). The dome was placed in the shade of an oak tree to minimize variation in instrument temperature. Further, the dome provided protection from the resident cattle. Power was provided by a 2kW portable gasoline generator which had to be refueled three times during the day of the experiment. A weather station was installed on a 3 m pole 40 m from the dome, clear of the oak canopy. Light received from a 95 m long observational path was collected by a ø 203 mm Newtonian telescope (Bresser, Location **T**, Fig. 2), focal length 800 mm, installed on a motorized equatorial GoTo mount (LXD75 Meade; the position was kept fixed during this experiment). The telescope's field of view (FOV) was directed towards a 1 m cubic box of black anodized aluminum 95 m straight north from the dome (Fig. 2, location **B**). We refer to this as the black termination, in the ideal case the termination is entirely dark in which case the only contribution to the light intensity received by the telescope would be the scattering from atmospheric constituent in stretch **T-B**, See Fig.2. The first 55 m of the FOV was over grassland and the remaining 40 m was over the river Klingavälsån. The FOV descended roughly from 4 m to less than 0.5 m over the river surface. Most of the FOV was illuminated throughout the day. The focusing and overlap of the FOV with the black termination was done with an imager in the focal plane, after which the telescope was locked in position and alignment. The imager was removed and a 1 mm UV fiber (Edmunds Optics) was instead installed at the same place as the image of the black box in the focal plane of the telescope. The FOV at termination was ø 120 mm, and the total air volume monitored was roughly 4 m^3. The other end of the optical fiber was fed to a compact spectrometer (Ocean Optics, USB4000). The spectrometer had a cylindrical lens and higher order rejection filters installed. The slit-width was 100 μm yielding 4 nm resolutions FWHM. The spectral region covers 345 - 1040 nm. The integration time was set to 20 ms throughout the experiment and thus 50 spectra were recorded per second. The data were stored by two laptop computers, one logging the data from the weather station and another one storing the data from the spectrometer on an external USB terabyte storage device. The cost of all equipment was roughly 5K USD, two magnitudes lower than in [1]-[5]. The spectrometer records a dark spectrum of the black termination unless something within the FOV scatters the sunlight into the telescope. The sunlight impinges on the FOV at roughly 58° at noon and at roughly 90° at six am and pm. However, the total atmospheric air mass prior to the intersection with the FOV was larger in the morning and evening. The experiment was carried out on the 3rd of July, with clear sky during the whole day. Recording started at 6:31am and finished at 5:25pm. All times stated throughout this paper are true sun times (*TST*), corrected for summer time settings and the longitude between the study site and Greenwich. In order to estimate the intensity and spectral profile of the illumination impinging on the FOV, standardized ø 30 mm pieces of white polystyrene foam were dropped through the FOV every hour. Precaution was taken so that the shadow of the operator did not disturb the white calibration. The calibration was performed at location **C** in Fig. 2.

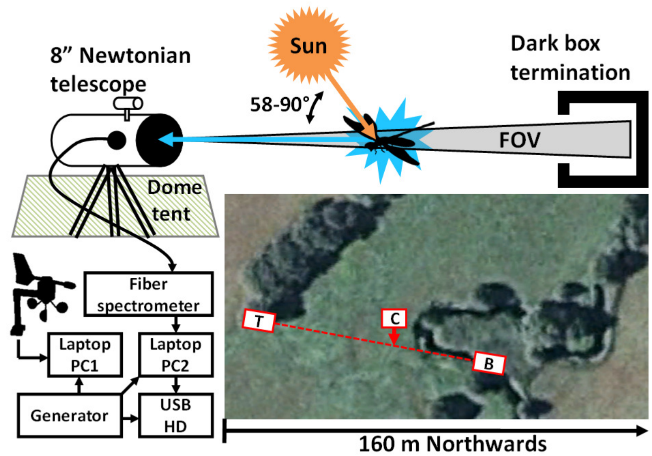

Fig. 2. Setup for the experiment. T: Newtonian telescope. C: Calibration site, B: Dark box termination. Light is collected by a 1mm UV fiber in the focal plane of the telescope and fed to a compact spectrometer. Distance from telescope to termination was 95 m of which the first 55 m cover grassland and the remaining 40 m cover the river Klingavälsån.

IV. Data Processing and Analysis

A. Temporal Detection of Rare Events

Initially, the intensity counts from each spectrum were averaged within the visible range 400 - 680 nm. The NIR region was omitted in order to avoid vegetation induced bias. The intensity vector over time, I_{Vis}, was filtered with a median filter with a time window of 2 s (101 samples); see Fig. 3. The filtered vector represents the static intensity counts, I_{Stat}, that arise due to three factors; from the dark-current (instrument temperature dependent), the non-perfect black termination, and from atmospheric scattering in the FOV, which can change during the day. The static background vector is subtracted from the intensity vector, so that a background corrected intensity vector is obtained, I_{BC}.

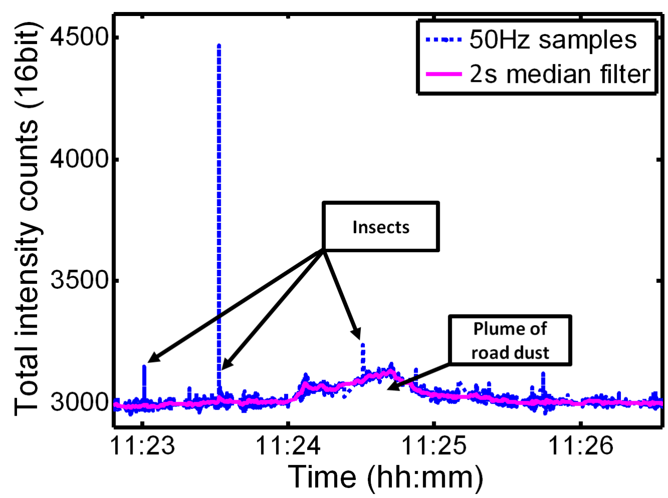

Fig. 3. The total scattered intensity over time, in the visible range, sampled at 50 Hz and with a 2 s broad median filter applied to the same signal, which is used to estimate the static signal. The positive fast spikes are caused by insects. Typically, the specimen passes the FOV in less than 50 ms. For comparison, the slow increase of scattering between 11:24 and 11:25 arose from a wide dust plume that was caused by a tractor driving upwind.

A threshold is then used to detect all rare events that are caused by insects intersecting the FOV. Since the intensity impinging on the FOV, I_0, changes during the day, the threshold is weighted by I_0. We describe the time dependent I_0 as follows:

$$I_0(TST) = A\left(\frac{1-\cos(\frac{\pi}{12}TST)}{2}\right)^w + D \qquad (1)$$

The parameters *A*, *W* and *D*, were fitted to the I_{BC} for white reference events carried out throughout the day. *A* represents the light intensity and instrument sensitivity, *D* represents a bias and *W* changes the waveform of the daily cycle. By plotting the skewness of I_{BC} on a histogram (Fig. 4), the noise level can be estimated from the negative values of I_{BC}, and a level where the positive spikes can be identified with a negligible risk of including a false positive. The time dependent threshold was scaled by this level. 1526000 spectra were recorded during the day; and 3613 of these spectra were identified to belong to scattering events caused by insects given the specified threshold. When merging consecutive scattered spectra, the number of insect events passing the FOV was 1285. The average chance of detecting an insect for each acquisition was thus $2.4 \cdot 10^{-3}$, and if we assume no interactions between insects, then the risk for pile-ups would be $5.6 \cdot 10^{-6}$ which is negligible; however, such an assumption is arguable. As can be seen in Fig. 4, a large number of events were also caused by insects below the threshold. These can also be identified when observing I_{BC} over time. They could be caused by smaller insects, which could potentially be prey species upon which the damselflies are feeding [6]. These events are omitted from this study, since their signal to noise ratio (SNR) would be too low for spectral analyses.

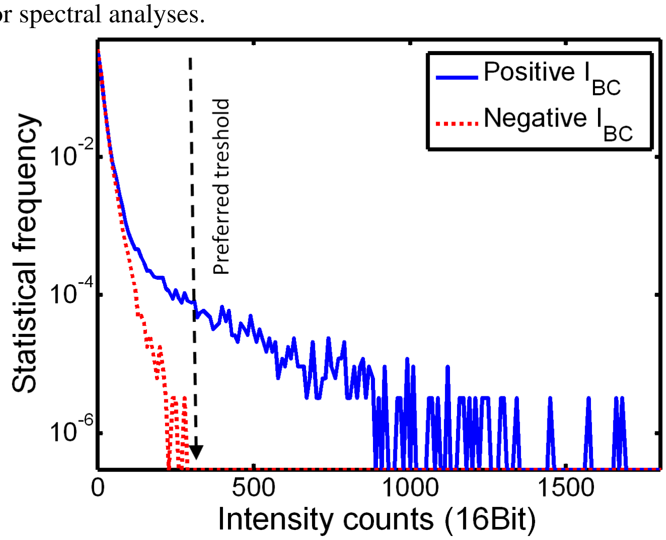

Fig. 4. Statistical frequency per sample in the late afternoon around 4:13 PM *TST*. The negative part has been flipped into a positive one for comparison. The obvious skewness arises from the rare events caused by insect intersecting the FOV. The negative observations show the noise levels and were used to set the threshold in order to avoid false triggering.

B. Spectral Processing

The full spectra from the triggered events were extracted from the data files, and the neighboring static spectra were subtracted in such a way that dark current, imperfect dark termination and slow atmospheric scattering were rejected so that only the scattering contribution was considered. In order to compensate for the spectral profile of the sun's emission, the spectral throughput of the atmosphere, the telescope, the fiber and the sensitivity of the spectrometer, all scattering spectra were divided by the scattering spectrum from standardized white polystyrene foam pieces. The part of the spectra with reasonably good intensity (380 - 900 nm) was decomposed linearly using singular value decomposition (SVD) [18]-[21]. The singular values showed unambiguously that all scattering events during the entire day could be explained as a linear combination of 6 base spectra (see Fig. 5), thus we are able to represent each spectra with 6 variables rather than one for each for the original 2664 spectral bands. A 6-dimensional color space was expanded by this set of orthogonal base spectra and the scores were weighted by the singular values to maintain equal SNR on each axis. Because the absolute scattered intensity is likely to vary with the FOV-specimen-overlap and the cross section with the orientation and wing beat phase, the six scores were divided by the first principal component score. This kind of auto-normalization leaves us with a spectral shape for improved spectral classification [22]. Further, since the first axis always becomes 1, we can reduce the dimensionality of our representation by 1, and thus we can now describe the spectral shape of each event in a 5-dimensional color space.

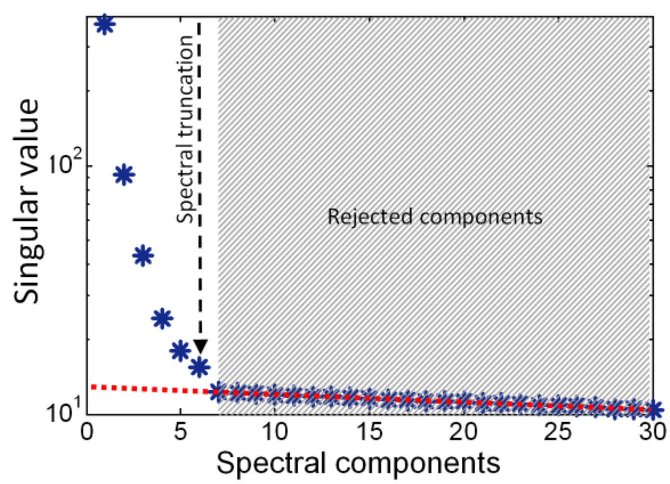

Fig. 5. When decomposing the spectra of the 2664 spectral bands of the 3613 events, the singular values suggest a remarkably clear truncation point after six spectral components. The red dashed line indicates the noise floor. Projection of data onto the first component gives a SNR of 30:1, whereas projection onto the sixth component only yields a SNR of 2:1.

C. Unsupervised Clustering and Classification

After redundant information was removed with SVD, the Euclidean distances, in the 5D color space, between each event and all other events were calculated, and the distances were fed into a hierarchical cluster analysis considering the furthest distance (see Fig. 6) [23]. The number of clusters should be at least as many as the spectral truncation number; otherwise the spectral components would not be independent. However, the number of clusters can be larger than the number of spectral components. This would be the case if several clusters share the same chromophores, but in different discrete concentrations [1], [2]. The four groups of damselflies (males and females of *C. splendens* and *C. virgo*) share the same melanin pigment, but are differently melanised in discrete quantities [3],[24]. The most prominent signature, however, arises from the structural phenomenon and this does not decompose linearly because the center wavelength and feature width relates to the size and ordering of the nanostructures, respectively, rather than to specific spectral transition energy. Another spectral component, which is unrelated to the damselflies, arises from light reflected off the surrounding vegetation and carries the imprint of the chlorophyll absorption. This feature is mainly characterized by the steep slope at 700 nm. This component can be expected to be much stronger in events detected over grassland (the first 55 m of the FOV) in comparison to events detected over the river (the last 40 m of FOV). Since this component reflects in any event, the effect would be a multiplication by two of any original discrete clusters, i.e., each cluster with and without a vegetation imprint.

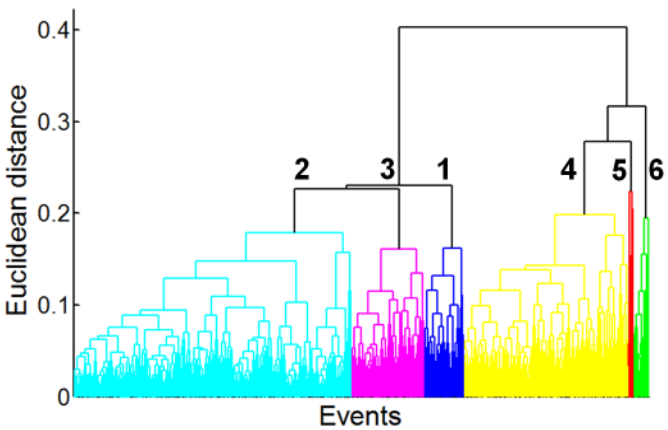

Fig. 6. The Euclidean distance between each event represented in the 5D color space is fed into a hierarchical cluster analysis. This dendrogram shows how similar the 3613 events are to each other. The first 6 branches were interpreted.

We interpreted the first 6 branches of the hierarchical tree by plotting the cluster centroid spectra. The centroid spectra were calculated as the mean of all spectra assigned to a particular cluster (see Fig. 7). Clusters 3, 4 and 5 show characteristic blue features, which are unlikely to arise from anything else natural other than the nanoarrays of damselfly males. The blue feature has close resemblance to the laboratory reference measurements in [3]. We are uncertain, however, whether the several peak positions (see cluster 3-5 in Fig. 7) relate to natural variance, the age of the individual, or perhaps the fact that two species are present at the site. Cluster 3 shows a significant imprint of vegetation, whereas cluster 4 and 5 can be assumed to be associated with males flying over the river surface. Cluster 1 and 2 can be associated with damselfly females. The green spectral feature at 550 nm could potentially be of importance for crypsis when the damselflies sit in the vegetation. This feature is less intense and broader than for males, as previously shown in laboratory measurement [3]. Cluster 2 carries a significantly larger vegetation imprint than cluster 1; and for this reason cluster 1 is associated with females flying over the river and cluster 2 is associated with females flying over the grassland. To further strengthen the statements made above, we can attend to a small detail in the centroid spectra presented in Fig. 7. The oxygen absorption band at 760 nm is a Fraunhofer line from the earth's atmosphere. The oxygen path length from the detector to the insect compared to the several kilometers path length prior to the incidence on the FOV is basically insignificant; however, the steady state fluorescence of vegetation fills up this Fraunhofer line [25]. The white calibration was performed over grassland; thus the oxygen line is not present in the two clusters that carry vegetation imprint, namely cluster 2 and 3. In cluster 1, which contains events over the river, the lack of vegetation fluorescence at 760 nm leaves a minor dip around 760 nm. Cluster 6 is presumably related to the darker male damselflies that are characterized by a less prominent structural feature; however, we prefer to refer to this cluster as uncertain. The centroid spectra presented in Fig. 7 can be thought of as attractors in the 5D color space, and each recorded scattering spectrum would fall into the cluster which it resembles the most. By analyzing consecutive scatter spectra, the consistency could be assessed. It was found that 80% of all consecutive scatter spectra were classed to the same cluster, and 93% were classed to the same sex.

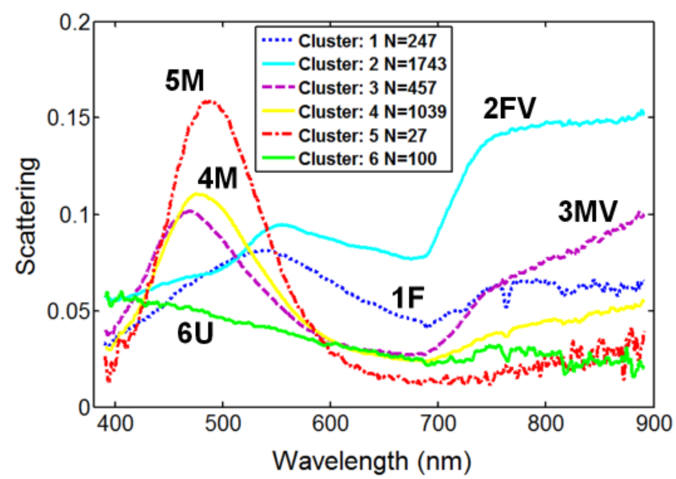

Fig. 7. Mean scattering spectra of each cluster. A scattering coefficient of 1 corresponds to the scatter from a white calibration polystyrene foam piece. M: Male, F: Female, V: Vegetation imprint, U: Uncertain. The spectra were interpreted as follows: 1F: female, 2FV: female with vegetation imprint, 3MV: male with vegetation imprint, 4M: male, 5M: male, 6U: Uncertain.

V. Biological Results

A. *Interactions between sexes*

A common idea is that males chase females when they arrive at the river for reproduction [6]. If we interpret 'chasing' as two insects sharing the same trajectory in space, where the second insect is somewhat delayed behind the first, then there should be an increased chance of spotting a male when a female is detected if this hypothesis is true. To test this, we estimated the time dependent correlation [26], [27] between the females classed to cluster 1F with the males classed to cluster 4M and 5M (Fig. 8). The symmetric female autocorrelation is plotted with a green line for reference. This autocorrelation shows the duration of the scattering events arising when the female damselflies intersect the FOV. The male autocorrelation is plotted with a red line. From the width of the male autocorrelation, it can be seen that males stay longer in the FOV. In addition, the shape of the female- and male autocorrelations differ. While the autocorrelation at 0.5 is 54 ms for females and 70 ms for males, at 0.1 the difference in width of the autocorrelations has increased considerably to 120 ms and 248 ms for females and males, respectively. This could potentially be due to the territorial behavior of the males, which causes them to patrol and/or engage in territorial male-male interactions with con- and heterospecifics [28]. Alternatively, their flight patterns could differ with respect to the monitored path, e.g. the case of males mainly flying along the river. To our surprise, we find a female-male cross correlation, where females appear in the FOV approximately 100 ms after males have passed. This is likely the result of pre-copulatory tandems between males and females, rather than females chasing males. In such tandems, males grab females by the thorax with their clasping organs. When flying, clasped females are then connected to the males in such a way that they have to fly behind the males and hence pass the FOV after the males. We also find a much weaker, but still significant, correlation where males follow females, however. This less-well defined increase in males that enter the FOV approximately 1 second after females have passed supports the prediction that females flying over the river are chased by males. The dashed confidence line on the bottom of the plot shows the 99% confidence limits of the female-male long term cross-correlations for the entire day. This type of analysis, with inspiration from digital signal processing [29] and system identification in robotics [30], is not possible with manual observations because of the poor time resolution, large uncertainty and short observation duration. Clearly, a longer recording time would improve the accuracy of these types of correlations.

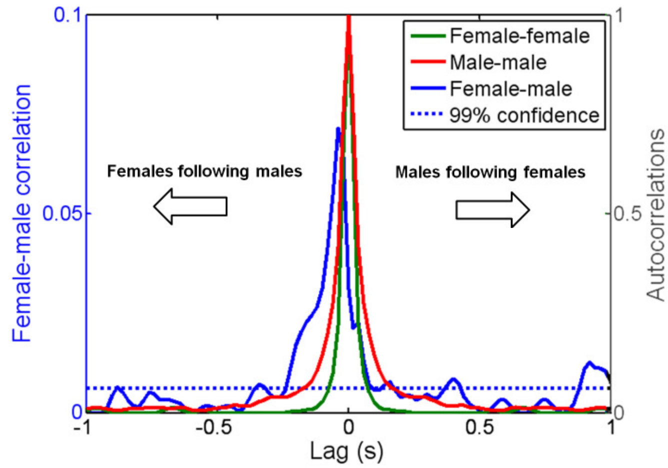

Fig. 8. The males classed to cluster 4M and 5M were cross-correlated with the females classed to cluster 1F. Positive lags on the female-male correlation correspond to males being spotted after a female spotting. The dashed confidence line is valid for female-male correlation. Note the different scales on the Y-axis.

B. Activity patterns in relation to time, temperature and wind speed

The activity (in terms of number of damselflies in the FOV per hour per cubic meter) is similar to that estimated by manual censuses (Fig. 9). The relative proportions of male and female damselflies are also consistent (Fig. 9), which supports that the clusters have been correctly classified. The damselfly densities are also suitable for analyses of patterns on a much slower time scale than shown in Fig. 8. The activity of the two sexes can, for example, be analyzed with respect to the true sun time, wind and temperature (Fig. 10).

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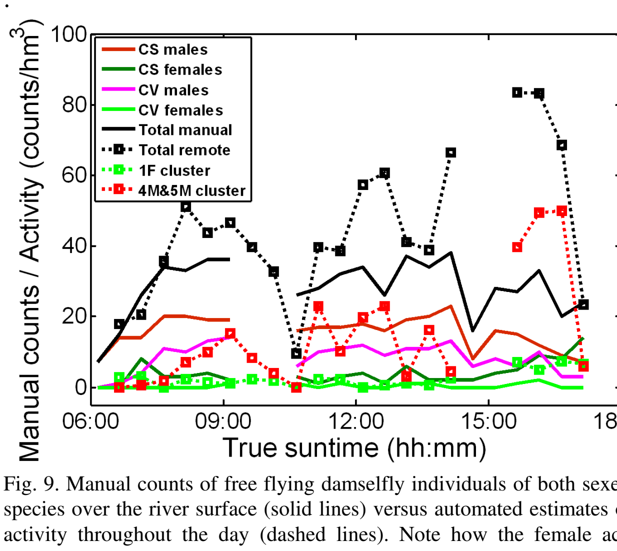

Fig. 9. Manual counts of free flying damselfly individuals of both sexes and species over the river surface (solid lines) versus automated estimates of the activity throughout the day (dashed lines). Note how the female activity increases towards the end of the day for both manual counts and the automated method.

In general the activity of organisms with respect to temperature can be described by the shape:

$$Act(T) = Act_0 \sqrt{T_{\max} - T}\; e^{-\frac{T_\alpha}{T - T_{act}}} \qquad (2)$$

Where *Act* is the activity in counts per hour cubic meter, T is the environmental temperature, Act_0 is the general activity related to other circumstances than temperature, T_{max} is the maximum temperature that the organism can be active at, T_{act} is the minimal temperature at which the organism can be active, and T_α is the thermal sensitivity. This model is, however, hard to excite [1] outside a laboratory environment where the temperatures cannot be manipulated over the entire range. Instead, we apply a simple and more robust second order polynomial thermal relation with one less degree of freedom (DOF). We also include a term allowing the flight preferences to depend on wind speed. Because the gradient of such a relation must necessarily be continuous and zero at zero wind speed, only even polynomial terms of the Taylor expansion of the wind parameter can be included:

$$Act\,(T\,,v) = k_0 + k_{T1}T + k_{T2}T^2 + k_v v^2 \qquad (3)$$

Here v is wind speed in meters per second, and *k's* are model parameters found by multidimensional regression with the QR factorization. Clearly, the accuracy, model excitation, details and DOFs of the model can be increased if longer recordings are performed. We have not included an analysis of wind direction with respect to the orientation of the FOV in this study, even if this could have implications for the observations.

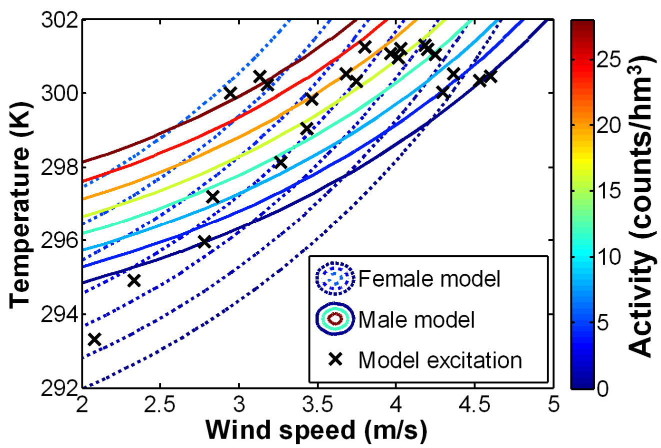

Fig. 10. Contour lines of flight preferences of both sexes based on computerized measurements. Female observations are plotted with dashed lines, while male observations are plotted with solid lines. As in Fig. 8 the absolute activity of the females is lower than that of the males, implying that they do not reach the top levels of activity, due to a lower number of females flying over the water surface.

VI. Discussion and Outlook

The idea of this study is not to provide highly accurate data on the focal species[2], but rather to explore new opportunities for using automated and modern electro-optics and spectral classification in ecological monitoring. We have successfully demonstrated remote insect classification using an inexpensive, portable and passive setup. One clear advantage of spectral identification in comparison to spatial image identification is that spectra do not become blurred when the sample is not in the focal plane of the telescope, and it does not produce a fast moving induce motion blur as is does in the spatial domain [31]. Clearly, the accuracy and reliability of the analysis of ecological events would benefit from longer recording times to improve statistical power.

The setup enabled us to successfully identify the two damselfly species to sex from their dark field scattering differences. Since the classification presented in this study is unsupervised and we only had reference scattering spectra for the two sexes of *C. splendens* [3], we choose not to interpret more than the first six branches in the dendrogram in Fig.6. For this reason, we cannot exclude the possibility that a refined analysis of the lower branches would enable us to discriminate between the two species. Although the coloration of the abdomen is fairly similar in the two species, the melanization of wings differs considerably (Fig. 1). The proportions of males and females as well as the damselfly densities that were measured with our setup were generally consistent with the manual counts (Fig. 9). This suggests that the setup used in this study does obtain reliable counts and activity patterns that can be used instead of the more laborious and time consuming manual techniques. In addition to this, our measurements clearly showed a vegetation signature in the spectra of the categories 2FV and 3MV (Fig. 7). These groups are probably made up of damselflies that were flying above vegetation rather than over the river. Obtaining these habitat measures (e.g. river versus grassland habitats) simultaneously with the damselfly counts is a particularly promising part of this method, because it allows, in addition to population monitoring, ecological insights into the lifestyle, activity patterns and possible interactions between individuals.

Another biologically interesting result is the correlation between males and females (Fig. 8). Females are more likely to be found at a well-defined and very short time behind males. This phenomenon is most likely caused by pre-copulatory tandems, in which the male has clasped the female. We also see a lower and less-well defined signal of males following behind females at approximately ten times longer lag time. This implies that males chasing females typically appear at ten body lengths distance after the females. In the courtship interactions males are commonly observed chasing the females during manual counts, and our results suggest that a common chasing distance is approximately ten damselfly body lengths. More information on the flight distances, directions of the chase in relation to the telescope, and flight speed would be needed to draw any further conclusions from this data. Nevertheless, although more fine-tuning is needed, we have shown that it is possible to study the interactions between insects over very short timescales. This technique can be applied to study both interactions within- and between sexes of the same species, such as territorial behaviors and chasing of potential mates, and to study interactions between species, for example predator-prey interactions. Finally, both males and females

[1] In system identification, *model excitation* implies the region for which the model can be trained.

[2] In biology, the *focal species*, refers to the species described in the study.

were more active at higher temperatures (e.g. activity increased from 292 K to 302 K which was the highest temperature during the experimental period) and in lower wind speeds (activity was highest at 2 m/s, which was the lowest wind speed during the experimental period, and activities clearly decreased towards wind speeds of 5 m/s). Interestingly, there was a slight difference in the temperature dependence of the different sexes where females were more active at slightly lower temperatures whereas males remained more active at slightly higher wind speeds (Fig. 10).

Although the measurements with this experimental setup were rather successful, a number of improvements could be carried out: first, the analysis would benefit from an improved SNR. The components used in this experiment were simply the ones available in our laboratories and were not necessarily the optimal choice for the experiment. Given the SNR, the broad spectral features and the necessity for a fast sampling, a slit-width should be chosen that allows an increase of light. The SNR could further be improved by choosing a detector and grating blazing angle for optimal sensitivity around 400 nm. This would allow exploitation of the 300-400 nm region, where several interesting features could be expected (for example absorption of chitin below 330 nm and UV-features visible to the focal species and their bird predators). In the present experiment the blaze was optimized for NIR, and the obtained signal was too weak for the UV region, even if plenty of sunlight can be found in this region. Thermoelectrically cooled compact spectrometers would further improve the SNR. Even compact multichannel photo multiplying tubes (PMT) spectrometers could be considered [32]; however, the costs are considerable, the channels are few, and they typically do not include higher order rejection filters, which is necessary for such experiments. Improved SNR would also allow the detection and monitoring of smaller insects, and for example, the interaction between predator- and prey insects could also be studied. While the damselflies in this study have rather slow and chaotic wing beats, detection of other species might benefit from even faster sampling rates towards kHz [33], [34]. For studies of this kind, PMT's or Avanlanche Photo Diodes (APD's) should be used.

An improved black termination with improved shielding from the sunlight incident from all angles throughout the day would further make the interpretation easier and cancel out some uncertainty. Also, the white calibration could be made more consistent; one way would be to use a white sphere on a motorized swinging axis, so that the timing and intersection with the FOV is identical during each calibration event. Reference measurements of several groups of species and sexes could also be performed for improved interpretation of the centroid spectra.

For long-term unsupervised recordings, several issues should be addressed. A more robust telescope mount is necessary; also the entire setup would need a box or shed for weather protection. A CMOS RGB all-sky-imager should be included to monitor the sky in relation to the illumination of the FOV, which might be uneven on a partially clouded day. The USB storage device should preferably be powered by laptops, so that the recordings are not interrupted by power cuts. The generator should preferably be exchanged with a solar panel.

A number of problems and questions still remain unsolved. Those are the estimation of the sample-FOV overlap, estimations of range to the events and direction of flight with respect to wind direction. Also, discrimination between the two species would be desirable to allow the more detailed investigation of the interactions and general ecology of them. One of the most interesting techniques involves the marking of individuals without recapture, such as those previously done with active fluorescence LIDAR [4], [5]. These studies allow the estimation of insect dispersal and lifetimes. This was not pursued in this study, but is likely to work well in the passive mode.

Although we describe purely passive sensing in this paper, there might be several benefits of intermittently employing an inexpensive continuous wave (CW) laser diode (LD), available from 405-980 nm up to 1W power [35]. The method could still be non-invasive and non-perturbing if a NIR LD is used. Potential benefits could include the possibility to estimate the distance by $1/r^2$ attenuation, and to estimate the sample-FOV overlap. The latter could be solved by painting the termination with a special fluorophore responding to that laser line with a given signature. Then, any obstruction of the beam would cause a decrease in that spectral component. Rare earths would produce very specific signatures, and could be used for various encoding strategies. Even fluorescent marking experiments, without range resolution, could be performed.

VII. Conclusion

We have presented a simple, compact, inexpensive, and portable setup for remote insect classification. In contrast to earlier LIDAR based studies [1]-[5], this setup can easily be employed by biologists. In the present study, we have also provided an outline of how data from such a setup can be analyzed, and have given some tentative ideas that could be addressed with such data. We presented quantitative measures of temporal variations in damselfly activities during a day cycle, and related activities to temperature and wind speed. Moreover, phenomena were presented on a fast (millisecond) timescale, which has not been possible to estimate previously. Finally we have discussed possible improvements and perspectives for future studies.

TABLE I
VARIABLES AND ABBREVIATIONS

Short form	Explanation	Comments
FOV	Field of view	Roughly 95 m long, 4m^3 air volume
I_{Vis}	Intensity counts in the region 400-680 nm	Ranges from 0-65536 (16bit), includes dark current, imperfect termination, atmospheric scatter, insects scatter
I_{Stat}	Quasi-static intensity,	Includes dark current, imperfect termination, atmospheric scatter
I_{BC}	Background corrected intensity counts	Includes insects scatter, centered around zero with a considerable skewness
FWHM	Full width half maximum	Given by the fix slit width of the spectrometer
I_0	Time dependent intensity impinging on the FOV by which the trigger threshold is weighted	This function compensates for the increased light at noon
A, W, D	Model parameters for I_0	Fitted from white calibration events
TST	True sun time	Time corrected for summer time and longitude
SNR	Signal to noise ratio	Estimated from the noise floor of the Eigenvalues
Act	Insect activity	Measured in counts/hm^3
v	Horizontal wind speed	Measured m/s approximately 5 m over the river surface
T	Local temperature	Measured in Kelvin
$T_{max.}T_{act}$	Model parameters for thermal dependence	
$k_0..k_v$	Model parameters for flight preferences	Fitted individually for both sexes using the slow time statistics
DOF	Degrees of freedom for statistical models	
VIS	Visible region	400-680 nm in this study
UV	Ultraviolet region	300-400 nm in this study
NIR	Near infrared region	700-1100 nm in this study
SVD	Singular Value Decomposition	Method for removing spectral redundancy
QR	QR factorization	A rapid method for solving the least square problem in linear algebra
CW	Continuous wave	
LD	Laser diode	
PMT	Photo multiplying tube	
APD	Avalanche photo diode	

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