1	Optical remote sensing of submerged aquatic vegetation: opportunities for shallow clear water
2	streams
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13	analysis, very high resolution image data
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15 Abstract

Remote sensing has rarely been used as a tool to map and monitor submerged aquatic vegetation (SAV) in rivers, due to a combination of insufficient spatial resolution of available image data and strong attenuation of light in water through absorption and scattering. The latter process reduces the possibility to use spectral reflectance information to accurately classify submerged species. However, increasing availability of Very High Resolution (VHR) image data may enable the use of shape and texture features to help discriminate between species by taking an Object Based Image Analysis (OBIA) approach, and overcome some of the present limitations.

This study aimed to investigate the possibility of using optical remote sensing for the 23 detection and mapping of SAV. It firstly looked at the possibilities to discriminate submerged 24 macrophyte species based on spectral information only. Reflectance spectra of three macophyte 25 species were measured *in-situ* across a range of submergence depths. The results showed that water 26 depth will be a limiting factor for the classification of species from remote sensing images. Only 27 Spiked Water Milfoil (Myriophyllum spicatum) was indicated as spectrally distinct through ANOVA 28 29 analysis, but subsequent Jeffries-Matusita distance analysis did not confirm this. In particular Water 30 Crowfoot (Ranunculus fluitans) and Pondweed (Potamogeton pectinatus) could not be discriminated at 95% significance level. Spectral separability of these two species was also not possible without the 31 32 effect of an overlying water column.

Secondly, the possibility to improve species discrimination, using spatial and textural information was investigated for the same SAV species. VHR image data was acquired with a Near Infrared (NIR) sensitive DSLR camera from four different heights including a telescopic pole and a Helikite UAS. The results show that shape and texture information can improve the detection of the spectrally similar Pondweed and Water Crowfoot from VHR image data. The best performing feature 'length/width ratio of sub-objects' was obtained through expert knowledge. All of the shape and texture based features performed better at species differentiation than the spectrally based features.

In conclusion this study has shown that there is considerable potential for the combination of
 VHR data and OBIA to map SAV in shallow stream environments, which can benefit species
 monitoring and management.

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44 Introduction

Collecting data on submerged aquatic vegetation (SAV) from fluvial environments, which sufficiently represent spatial variation along a river reach, is difficult to achieve and often requires destructive and labour-intensive fieldwork (e.g. Flynn et al., 2002). Methods to obtain information remotely could therefore be of great benefit to the field of river science, including ecohydraulics. However, a combination of insufficient spatial resolution of image data and strong attenuation of light in water through absorption and scattering has long been a barrier for the application of remote sensing technology to study fluvial environments (Gilvear et al., 2007; Marcus and Fonstad, 2008).
This paper describes a project that applies a set of novel remote sensing techniques to map SAV,
which could help overcome some of these limitations.

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55 Remote sensing has so far rarely been used as a tool to map and monitor submerged aquatic vegetation in rivers (Marcus and Fonstad, 2008). A recent study by Lee et al. (2011) is one of the first 56 to look at the feasibility of using airborne hyperspectral image data to map SAV communities in 57 rivers. They studied the separability of four vegetation types in small rivers in western Nevada, US, 58 which included submerged brown and green filamentous algae communities. In the UK Hill et al. 59 (2009) were some of the first to attempt estimating submerged vegetation biomass (Water Crowfoot) 60 from image data taken with an airborne hyperspectral sensor. They did this for the River Frome chalk 61 stream. Although a reasonable estimate could be made, the success of this analysis was severely 62 limited by the quality and spatial resolution of the data (>1m) (Visser and Hill, 2011). Clearly further 63 64 work is required in this field.

While for terrestrial applications light in the near infrared wavelengths (NIR) is particularly 65 useful for the detection of variation in vegetation cover, the absorption characteristics of water limit 66 its use for SAV. As a result of both absorption by water and scattering by particles, light is attenuated 67 68 with distance travelled through the water column. In the optical range of the electromagnetic spectrum 69 NIR is more strongly absorbed by water than the visible wavelengths (VIS). NIR can therefore only 70 be used in image data of very shallow aquatic environments ($< \sim 1m$) to provide any information about bottom features. It also means that in sufficiently shallow aquatic environments variation in recorded 71 NIR reflectance does not only reflect variation in vegetation types or condition, but also variation in 72 the depth of the plant below the water surface. When applying optical imagery to map SAV, this 73 results in an unfortunate situation, which is described by Hedley et al. (2012) as 'environmentally 74 75 limited remote sensing'. Variation in depth, variation in the reflectance signatures of the bottom 76 substrate or cover types and potentially other factors such as water clarity, together contribute to 77 overall variation in the signal recorded by an image sensor and can lead to an overlap between two or 78 more mapped vegetation classes.

79 Hedley et al. (2012) focussed on such environmental limitations in Australian marine 80 environments. Generally more work has been done on remote sensing of SAV in marine environments 81 and various attempts have been made to resolve complications of submerged situations. O'Neill et al. (2011) for example used information about submergence depth to adjust above water reflectance 82 83 spectra for attenuation influence, using the empirical water attenuation correction by Maritorena et al. (1994). O'Neill et al. (2011) had access to a depth dataset and managed to produce a 97% overall 84 classification accuracy for Eelgrass detection. Depth data of sufficient quality is however not usually 85 available and certainly not of the detail required for fluvial environments. Lidar and radar data which 86 87 work for terrestrial situations again do not (yet) perform well enough in submerged fluvial conditions (e.g. Wang and Philpot, 2007). Important progress is being made with the application of inversion of 88 modeling of bio-optical models (e.g. Dekker et al. (2011) for marine environment and Giardino et al. 89 (2012) for lacustrine settings). Legleiter and Roberts (2009) explored the potential of inverse 90 modelling with regards to accuracy and precision methods for fluvial environments, using data 91 simulated with a forward image model (FIM). They found they methods would be suitable for depth 92 retrieval. However, data and analysis techniques are still insufficient to successfully apply them in 93 fluvial environments. 94

The foregoing overview identified how a combination of insufficient spatial and spectral 96 97 resolution of available image data, has so far ruled out their use for studies of smaller rivers (width < 98 10m). However ongoing improvements of image data collection and image analysis techniques are finally changing this situation. Unmanned Aerial Systems (UAS), which are small, low-altitude 99 100 remote sensing platforms such as small fixed winged planes or mini-helicopters, are rapidly developing into relatively cheap and logistically flexible means to obtain Very High Resolution 101 (VHR) multi-spectral image data. When classifying a remote sensing image to obtain maps of 102 submerged environments (e.g. SAV or river bed morphology) VHR data has the advantage that it can 103

104 generate detailed information on aspects of shape, structure and texture of the target surface. Using 105 so-called 'Object Based Image Analysis' (OBIA) techniques this information can be incorporated in the image analysis process to improve an image classification originally based on spectral information 106 only (e.g. Van der Werff and Van der Meer, 2008 and Laliberté and Rango, 2009). While 107 conventional image analysis techniques derive information about the target spectral reflectance on a 108 pixel by pixel basis, OBIA first segments the image data into spectrally homogenous objects. For each 109 object it then quantifies feature values such as shape (e.g. roundness or length/width ratio), internal 110 texture and characteristics of adjacent objects (e.g. contrast to neighbouring object). When such 111 additional object feature values are included in the analysis algorithm they can considerably improve 112 image classification (e.g. Blaschke et al., 2011). 113

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115 The study aimed to establish application of remote sensing methods for fluvial environments 116 and better appreciate the inherent limitations as identified by Hedley et al. (2012). This was done by 117 meeting the following two objectives:

- 118 Determine the possibility to discriminate between three submerged macrophyte species based
 119 on spectral information only.
- Determine whether discrimination of the same species could be improved using spatial and textural information obtained from VHR image data.

123 Methods

124 Introduction

125 Statistically discriminating between surface (cover) types based on spectral information, lies at the basis of classification of remote sensing image data. For this purpose spectral information about 126 the cover types is usually obtained from a training sample of pixels in the image. If separability 127 128 between the samples is higher, cover types can be mapped from the image more reliably. In order to check whether classification is possible in the first place and to find the optimal wavelength bands (or 129 130 band combinations) to do this, a considerable number of studies have also investigated separability of sets of individual spectra from cover types measured in-situ (e.g. Vahtmäe et al., 2006; Karpouzli et 131 132 al., 2004; O'Neill et al., 2011; Lee et al., 2011). In this study a GER1500 hand-held field spectroradiometer was used to collect reflectance spectra in-situ from the three submerged 133 macrophyte species across a range of submergence depths. 134

Remote sensing image classification processing time increases with the number of spectral 135 bands associated with a pixel, so techniques to assess separability between classes usually involve a 136 reduction in data dimensionality. A range of techniques have been applied to determine separability of 137 cover types, based on samples of in situ reflectance spectra (e.g. Lee et al., 2011; O'Neill et al., 2011; 138 Adam and Mutanga, 2009). This suggests that there is no consensus on what method is most suitable. 139 This observation is confirmed by Adam and Mutanga (2009) and Yang et al. (2005). Here we use the 140 methodology of Adam and Mutanga (2009), who took a hierarchical approach to reduce the 141 dimensionality of their data before determining species separability. One-way ANOVA was used with 142 a post-hoc Scheffé test to determine for each wavelength band which macrophyte pairs were 143 significantly different. This was followed by Classification And Regression Trees (CART) analysis 144 (Breiman et al., 1984) to select the most suitable bands for species discrimination. 145

146 A trained observer will be able to distinguish between Pondweed and Water Crowfoot by looking at their photographs despite their similar green colour. Their interpretation or 'classification' 147 of the image will therefore involve more than the clustering of spectral values, as done in the first part 148 149 of this study. OBIA attempts to simulate these additional human cognitive processes in order to improve image classification based on clustering of spectral values only. Recent studies by Phinn et 150 al. (2012) and Urbanski et al. (2009) have shown the benefit of this kind of approach for marine 151 environments. The second part of this study therefore investigates the possibility to improve 152 discrimination of the same three SAV species from image data, using spatial and textural information 153 in addition to the spectral information. VHR image data for this part of the study is acquired with a 154

Near Infrared (NIR) sensitive DSLR camera. Images are taken from four different heights, in order to understand how the OBIA approach is affected by the scale of the image data. The platforms used to achieve this include a telescopic pole and a Helikite UAS.

158 Study sites

The field sites for this study were located along two UK chalk streams: the River Wylye in 159 160 Wiltshire and the River Frome in Dorset. These calcareous groundwater-fed streams were selected because of their exceptional water clarity and abundance of a range of macrophyte species. Most data 161 were obtained from the River Wylve at Steeple Langford where it flows through the Langford Trust 162 nature reserve in Wiltshire. Additional data was collected from a distributary of the River Frome near 163 Wool in Dorset. The sites were physically very similar, with a stream width of around 5m and a 164 maximum water depth at time of sampling of around 50cm. Although this study involves one 165 particular type of stream only, the techniques and issues discussed are likely to apply to a much wider 166 range of clear water streams with SAV and to some extent also shallow lake environments. 167

The study focuses on three macrophyte species commonly found in the chalk streams: Water 168 Crowfoot (Ranunculus fluitans), Pondweed (Potamogeton pectinatus) and Spiked Water Milfoil 169 (*Myriophyllum spicantum*). Water Crowfoot is a keystone species of high conservation value for chalk 170 stream environments. The habitats they form are protected under the European Union Habitats and 171 Species Directive (92/43/EEC) (O'Hare et al., 2010). Management of the species is therefore a trade-172 off between conservation and growth control for fisheries and flood management. Remote sensing 173 174 could make an important contribution to improved management practices. The other two species were 175 chosen because of their relative abundance at the field sites and because pondweed is spectrally very similar to Water Crowfoot, but structurally rather different, while the opposite is the case for Water 176 177 Milfoil.

178 Spectral measurements of submerged aquatic vegetation

179 To collect reflectance spectra from three submerged macrophyte species, measurements took place with a GER1500 hand-held field spectroradiometer over several days in late August and early 180 181 September of 2009 and 2010 at both field sites. Due to limited access to the river and limited availability of specific vegetation species at different depths purposive sampling was applied to obtain 182 submerged vegetation spectra at a range of submergence depths. To obtain spectra of vegetation 183 184 without water column influences multiple layers of vegetation were piled on black painted canvas. The GER1500 was held at nadir 50cm above the water surface or canvas. The instrument has a 3° 185 field of view so the area measured on the target has a 2.6 - 4.0cm diameter (depending on 186 submergence depth), which is assumed sufficient to obtain representative spectral information from 187 the dense vegetation stands. Sampling was carried out on cloud-free days within 2 hours of solar 188 noon. Spectral averaging of 10-30 spectra per sample was performed to ensure optimal signal-to-189 noise ratio. A white reference Spectralon calibration panel of 99% reflectance was used every 5 to 10 190 samples to offset any change in the atmospheric condition and irradiance of the sun. Reflectance was 191 192 calculated by dividing macrophyte radiance by radiance from the Spectralon surface.

193 ANOVA and CART Analysis species discrimination

To analysis species discrimination we used the methodology of Adam and Mutanga (2009). They took a hierarchical approach to reduce the dimensionality of their data before determining species separability. This first involves a statistical test of differences in mean reflectance values for all combinations of two macrophyte species at each measured wavelength (350 to 1050 nm):

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- H0 : $\mu 1(i) = \mu 2(i) = \mu 3(i)$
- 200 H1 : at least one $\mu(i)$ is different
- 201

where μ 1-3 represent the mean reflectance of the 3 macrophyte species and i denotes the spectral wavelength band. One-way ANOVA was used with a post-hoc Scheffé test to determine for each wavelength band which macrophyte pairs were significantly different. ANOVA was tested for 99% and 95% confidence levels (p < 0.01 and p < 0.05). For each wavelength band the number of significantly different macrophyte species combinations were counted (in this case max. 3) to determine the wavelength bands most suitable for spectral discrimination. The one-way ANOVA coupled with a post-hoc pair-wise comparison resulted in a frequency plot of statistically significant mean reflectance values for each wavelength.

Although significant difference for the ANOVA test indicates at which wavelengths species 210 are most likely to be spectrally different, it does not guarantee separability of the macrophyte species 211 based on individual wavelength bands from this region. A measure for correct classification of the 212 vegetation types from image data using a single or combination of multiple bands can be determined 213 by calculating Jeffries-Matusita (J-M) distance values. However, it is very time consuming to 214 215 calculate the distance measure for all possible combinations of the bands identified by ANOVA. Adam and Mutanga (2009) therefore performed a further step to select the most suitable bands for 216 217 species discrimination, using the Classification And Regression Trees (CART) approach (Breiman et al., 1984). CART is a form of binary recursive partitioning that permits accurate prediction or 218 219 classification of cases, using both continuous and categorical variables. Training data is used to identify 'splitting' variables based on an exhaustive search of possible variable combinations. 220 221 Repeated partitioning of the data with additional variables occurs until criteria for predictive accuracy 222 are met. This automatically results in the optimal number of bands for separation of all 223 classes/species.

For this study we did CART analysis using the bands from 99% confidence level regions as input and compared the results with CART analysis using the full set of bands to confirm the benefit of initial band selection through ANOVA. Each tree/model was validated with a test sample of at least 25%. Because we were particularly interested in the possibility to separate the spectrally very similar Pondweed and Water Crowfoot, additional CART band selection was performed including these two species only and the results will also be presented.

Finally Jeffries-Matusita (J-M) distance values were calculated for the wavelength band 230 combinations selected by the CART method. To determine to what extent improvement of species 231 232 separation was achieved at the different stages of the analysis process, we also calculated J-M values for 5 sets of 5 band combinations ranging from 2-6 bands which were randomly selected from the 233 234 ANOVA 99% confidence level regions only, as well as J-M values for 5 sets of 5 band combinations ranging from 2-6 bands selected at random from the 741bands included in the analysis. The square of 235 the J–M distance values ranges between 0 and 2, with larger J–M distance values indicating greater 236 separability between group pairs. Values greater than 1.9 indicate that the sample pairs have good 237 separability (ENVI, 2004). 238

240
$$J - M_{ij} = \sqrt{2(1 - e^{-\alpha})}$$

241

242 With 243

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$$\alpha = \frac{1}{8} (\mu_i - \mu_j)^T \left(\frac{C_i + C_j}{2}\right)^{-1} (\mu_i - \mu_j) + 2 \ln \left(\frac{\left(\frac{1}{2}\right) |C_i + C_j|}{\sqrt{|C_i|x|C_j|}}\right)$$
2

245

Where i and j are the two species compared; C_i = covariance matrix of the spectral response of i; μ_i = the mean vector of signature of i; T = transposition function; $|C_i|$ = the determinant of C_i .

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249 Collection of VHR image data

250 Next we tested the usefulness of a range of image object features such as shape and texture to distinguish between macrophyte species in shallow rivers. We focused only on the two spectrally 251 252 most similar macrophyte species Water Crowfoot and Pondweed. The analysis was applied to a set of four multi-spectral images, which included stands of both species. All images were taken with the 253 same camera from four 'platforms' at different heights above the water surface in order to evaluate the 254 255 applicability of shape and texture features for species detection across a range of scales. The platforms included a tripod located in the river near a bridge at 1.5m elevation (location: 'from tripod'), from a 256 257 bridge at 3m (location: 'from bridge',) from a telescopic pole at 5.4m (location: 'from pole',) and 258 from a Helikite UAS (a combined helium balloon and kite) at about 5m elevation (location: 'from 259 helikite'.).

Despite strong absorption of NIR light in water, spectral signatures of submerged 260 macrophytes measured with the GER1500 field spectroradiometer, indicated that light in these 261 wavelengths may be useful in image classification (Visser and Wallis, 2010). Initial inspection of 262 263 NIR images also showed that plant structure and shape features appeared more strongly pronounced in this wavelength region. Because sufficiently light-weight multispectral sensors suitable for small 264 265 UAS-s are not available yet, multi-spectral images have been created with a Fujifilm IS-Pro NIR sensitive DSLR camera on a layer by layer basis, taking repeated photos of the same location and 266 267 stacking these subsequently using GIS software. A NIR blocking filter was used on the camera to obtain Red, Green and Blue image bands, A VIS blocking filter was used to obtain a band covering 268 most of the NIR spectrum (R72) and a bandpass filter was used to obtain a narrow NIR wavelength 269 band round 710nm (NIR (BP1)). Figure 1 shows the filter transmission spectra and their specifications 270 271 are as follows:

- 272 <u>R, G, B:</u> MaxMax X-Nite CC1 NIR blocking filter (centre: 483nm; 50% transmission: 325nm, 645nm)
- 274 <u>NIR(R72):</u> Hoya R72 VIS blocking filter (<720nm)
- 275 <u>NIR(BP1):</u> MaxMax XNiteBPB band pass filter (650nm to 787nm; 5% low cut 5% high cut)

5-Band image composites were created by overlaying and rectifying the different wavelength bands based on manually located ground control points in each image. Parts of the scenes not covered by all image bands were cropped before further analysis. No suitable photos were collected with the NIR(BP1) filter from the Helikite platform, so this band is missing from the 'from helikite' image stack.

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283 Image segmentation and object analysis

284 To obtain image objects and enable calculation of meaningful feature values from these, all images were segmented in Trimble eCognition image analysis software (Trimble, 2010) at two levels. 285 A first segmentation level was created using the Red and NIR(R72) bands only, which was suitable 286 for delineation of vegetated areas. At a scale parameter of 200 groups of objects best followed the 287 outlines of the main vegetation patches, while individual objects fully delineated the majority of 288 smaller patches (\pm 25cm diameter). 'Shape' and 'compactness' parameters were chosen as 0.5 and 289 0.1, since at this level object delineation should be determined by both shape and spectral 290 characteristics of the data, while the shape of the objects should be able to take on any form (i.e. low 291 292 compactness). Next the image objects at this first level were sub-segmented at a second level to obtain 293 objects that delineated the more detailed structure of the plants. The same image bands were used at 294 this level, but a scale parameter of 20 and shape and compactness parameters of 0.9 and 0 were 295 chosen. The latter two parameters indicate that object delineation was mostly determined by its shape and could take on any form. For all images these segmentation settings resulted in the creation of 296 297 rather elongated sub-objects, clearly representing the 'hair-like' shape of some of the macrophytes (see image close up in Figure 2). 298

A large number of features are available in eCognition to describe the shape and texture of image objects and many more can be 'designed' by the user. Due to their large number, selection of the most suitable features to classify species can involve similar procedures as used for spectral band selection. However the user can also use expert opinion to select the most meaningful features based on visual interpretation of the image data. A combined approach was applied for this study. The following two features were developed based on expert opinion and thought to describe the structural difference between macrophyte species:

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1) Mean length/width ratio of sub-objects.

This feature value is obtained by calculating the length/width ratio for all level two objects and averaging these within each first level object. The value seems to quantify the presence of a 'hairlike' structure in particularly Water Crowfoot patches.

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2) Mean standard deviation red of sub-objects.

This feature value is obtained by calculating the standard deviation of pixel values for all level two objects and averaging these within each first level object. The value seems to represent a relatively strong spectral difference between the stems and leaves of the Water Crowfoot plants.

317 A further selection of features for this analysis was taken from a range of texture measures that are standard included in the eCognition software. The eCognition 'Feature Selection Tool' used 318 for this purpose determines the most suitable features from a given selection. This resulted in a 319 selection of three Grey Level Co-occurrence Matrix features (GLCM), which are texture measures as 320 described by Haralick et al. (1973). The measures quantify the amount of variability between adjacent 321 pixels that make up an object. In this case the measures for contrast, dissimilarity and homogeneity 322 323 were tested. The various types of texture features and their different calculation methods tend to produce strongly correlated values and are likely to result in similar difference estimates. 324

For the objects representing each of the species the following range of feature values were calculated and exported into SPSS for further difference analysis:

- 329 Mean Length/ Width Sub-objects
- 330 Mean standard deviation red of sub-objects
- 331 GLCM Contrast (quick 8/11 all dir.)
- 332 GLCM dissimilarity (quick 8/11 all dir.)
- GLCM homogeneity (quick 8/11 all dir.)
- 334

In addition to this the average reflectance values for the objects in each band were calculated, exported and compared in the same manner. Because the number of Pondweed objects for some images were relatively small, a non-parametric Mann-Whitney U test was executed to determine to what extent there was a significant difference between object feature values of each macrophyte species.

341 **Results**

342 Spectral species discrimination

Table 1 shows a summary of the sample numbers and depth ranges measured for each of the macrophyte species. Each sample has a spectral range of 350 - 1050nm and a sampling interval of 1.5nm. An example spectrum is shown in Figure 3, which also shows the attenuation coefficient of water (K_d). Suspended load is mostly absent from the sampled streams, so no water quality adjustments were made.

The results of the ANOVA analysis for the submerged vegetation spectra and those of vegetation put onto the canvas are presented in Figures 4 and 5. The dark grey histograms indicate the

350 wavelength ranges where significant differences were found between combinations of two or more different macrophyte species with a 99% confidence level. The light grey parts of the histograms 351 indicate differences at 95% confidence level. The ANOVA test resulted in a slightly narrower range 352 of suitable wavelengths for the submerged vegetation spectra compared to those estimated for 353 vegetation taken out of the water. For submerged spectra differences at 95% significance level were 354 only found for the 500 to 600nm and the 850 to 950nm wavelength regions (Figure 4). For 99% 355 significance level this range was reduced to a region of visible green light between 525 and 576nm 356 and a very narrow section of the IR between 913 and 926nm. The latter finding is quite remarkable as 357 the IR wavelengths are expected to be strongly affected by water absorption. The range of significant 358 spectra measured on the canvas is wider (Figure 5), but considerable areas with too much overlap 359 between species remain. Similar to the submerged spectra significant differences are found in the VIS 360 wavelengths between 500 and 600nm. However, the range of significant NIR bands is different. 361 Significant wavelengths for spectra on canvas start at the beginning of the red edge (from 691nm) and 362 become less pronounced from 823nm onwards. The secondary y-axis in both figures indicates the 363 364 number of significant species combinations for each grey wavelength region. For both sets of spectra only significant differences were found between Milfoil and Pondweed or Milfoil and Crowfoot. 365 Differences between Pondweed and Crowfoot were not significant at 95% for any of the wavelengths 366 367 both above and below the water surface.

Table 2 shows the combined results of the CART and J-M analysis for the submerged 368 369 vegetation spectra. The table lists the J-M distance values from band selection resulting from CART as well as the best performing combinations of 2 to 6 bands, which were randomly drawn from the 370 significant ANOVA range and the full data set. Results of CART analysis performed on Pondweed 371 and Crowfoot data only are also included. Best performing band combinations are highlighted. Table 372 3 shows the same information for the vegetation spectra measured on canvas. The J-M distance 373 analysis results show that despite the significant difference between species for individual 374 wavelengths in the ANOVA analysis, actual separability of the species is not necessarily possible. 375 Complete separability of Water Milfoil and either of the other two species is possible when the 376 spectra are measured without the influences of water. However, J-M values only get up to 1.44 for 377 separability between Water Crowfoot and Pondweed. The J-M values become lower when the 378 vegetation is covered by a variable water layer. Better results are achieved when the number of bands 379 380 used in the analysis increases, however, attempts using up to 6 bands did still not result in the recommended minimum J-M distance of 1.9. For 6 bands highest values of 1.87 were obtained for 381 Pondweed and Water Milfoil, both other combinations had lower values: 1.66 for Crowfoot and 382 Milfoil and 1.62 for Pondweed and Crowfoot. The latter value was obtained for a combination with 383 384 one band less.

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386 OBIA species discrimination

Figures 6A-D show the outlines of the first segmentation level of objects in each image taken 387 from the four different platforms. Objects representing Pondweed and Water Crowfoot were manually 388 selected and are outlined in white and grey respectively. Table 4 shows the total number of objects for 389 each of the two species available for analysis. Table 5 shows the significance levels of the Mann-390 Whitney U test for difference in object feature values between both macrophyte species, as observed 391 392 from each platform. More detailed information about the distribution of each sample is shown in the boxplots of Figures 7A-F. The data indicates that the best results for separating the macrophytes were 393 achieved with the highest resolution data. Objects from the image taken from a tripod just above the 394 395 water surface show significant differences for all object shape and texture features tested. The lowest p values were obtained for the difference test using some of the spectral band values only (i.e. red, 396 BP1 and R72+BP1). All lower resolution data show fewer significantly different object features. The 397 feature that performs best is the 'mean length/width ratio of the sub-objects'. The remaining texture 398 features all perform similarly. Figures 7 A-H show boxplots that illustrate the distribution of values 399 for a selection of 8 features (red, green, blue, BP1, R72, mean length/width ratio sub-objects, mean 400 standard deviation sub-objects and GLCM contrast). 401

403 **Discussion and implications**

404 Spectral species discrimination

The results showed that water depth will be a limiting factor for the classification of species from remote sensing images. Spiked Water Milfoil was indicated as spectrally distinct from the other species across the observed range of water depths with ANOVA analysis, but this was not confirmed by Jeffries-Matusita distance analysis. In particular Water Crowfoot and Pondweed could not be discriminated at 95% significance level. J-M distance analysis values confirmed these observations. The latter two species are spectrally so similar that they could not be discriminated without the effect of an overlying water column either.

Both submerged macrophytes and those taken out of the water on the canvas show significant 412 413 differences in the VIS wavelength range between 500 and 600nm. This range corresponds with useful bands found by O'Neill et al. (2011) who found that most marked differences between benthic classes 414 415 occurred in the green spectral range between 500 and 600 nm, which coincides with lower K_d values. It also coincides with the photosynthetic pigment absorption minimum between that lies between 555 416 and 565. The position of the significant NIR range is rather different for each of the two data sets. The 417 range including the red-edge, found for the spectra on canvas, corresponds with findings for most 418 terrestrial vegetation species, which show most variation around this region. The same NIR region 419 does not result in significant differences for the submerged spectra, which was expected considering 420 the high variability in this wavelength region due to the increased K_d. Remarkably however, a small 421 region of wavelengths between 850 and 950nm is selected in the ANOVA analysis and some of the 422 NIR wavelengths contribute to the combinations that result in best separability. For emergent species 423 Adam and Mutanga (2009) also identified several wavelengths in this part of the NIR as most useful 424 425 for discrimination between vegetation species.

426 The results indicate that for accurate classification of any of the submerged macrophytes more 427 than 6 wavelength bands will be required. Depth clearly has an influence here as for the spectra 428 measured on canvas sufficient distance values were achieved for two out of the three species combinations with three bands or less. Comparisons with results from other studies are difficult due to 429 430 differences in experimental set up, but Lee et al. (2011) managed to discriminate between most algal 431 species with as little as two bands despite attenuation from the overlying water column. The suitable wavelengths found in their study related to variations in colour and presence of unhealthy cellular 432 structures. They mostly fall outside the wavelength ranges found in this study, which is most likely 433 due to the rather different vegetation types that they looked at. To discriminate between Eelgrass and 434 associated bottom types in non-water corrected remote sensing images of much deeper marine 435 conditions (1-30m) O'Neill et al. (2011) needed ten bands of 4 nm bandwidth. In their analysis they 436 include spectral derivatives (R') and band ratios. Using the same data corrected for water depth they 437 only needed 3 bands, though a classification based on these bands turned out to be less accurate. Their 438 findings included bands covering the peak (R'566) and shoulders (500-530 & R'580) of the green 439 reflectance maxima. This corresponds with the findings of this study, which identified significant 440 ANOVA results in the green wavelength region and bands from this region were included in the 441 selections with the highest J-M distance values. Their data did not include wavelengths beyond 442 443 800nm, which have proven most effective in this study.

CART analysis applied to the spectra on canvas consistently selected the 711nm band to 444 separate between the 'green' and the 'red' (milfoil) macrophytes, followed by bands of blue light (460 445 - 480nm) to further separate between the two 'green' species. The latter bands fall outside the range 446 447 selected as significant with the ANOVA test. CART analysis applied to the significant wavelengths of submerged species produces better J-M values than CART applied to all wavelengths. The bands 448 selected in the latter case are also mostly from the green light region and IR wavelengths beyond 449 950nm. The highest J-M distance values were not achieved for band combinations selected through 450 CART and also not always for band combinations taken from the statistically significant regions. 451 Although distances were not calculated for all band combinations, this suggests that the combined 452

ANOVA and CART band selection method may not be suitable as a data dimensionality reduction method in this situation. To confirm this analysis should be repeated with other band selection methods.

456

457 *OBIA species discrimination*

458 The first part of the analysis showed how the macrophyte species Pondweed and Water Crowfoot are spectrally so similar that even without water column influences they are difficult to 459 distinguish. These results indicate that information other than spectral reflectance needs to be 460 incorporated in image analysis to enable accurate classification of these species. The subsequent 461 testing of difference between species based on a number of texture and shape features confirms the 462 potential to do so. The good performance of the 'length/width ratio of sub-objects' feature confirms 463 that our initial visual interpretation of the image data was good and that such expert knowledge can be 464 useful for species discrimination. The feature does however not perform very well for the images 465 taken from the highest platform. This could indicate that the possibility to use the length/width shape 466 feature to discriminate the species deteriorates at a less detailed scale. Sufficient resolution may be 467 needed to produce the more elongated object shapes for Water Crowfoot during segmentation. 468

In general contrast features performed well, which confirmed visual interpretation of the 469 images showing clear variation in spectral contrast amongst the two species. The spectral features 470 perform worst with some not allowing discrimination of the species at any scale (e.g. red band). This 471 472 result corresponds to some extent with the ANOVA test results which showed only narrow regions of 473 wavelengths with sufficient difference between the macrophyte species. The best performing bands however do not seem to correspond exactly with the significant wavelength regions (e.g. blue band). 474 475 The poor performance of the spectral features in general does support the original expectation that incorporation of shape and texture information is essential for successful classification of SAV. It is 476 477 however unclear why there is considerable variation in separability amongst the different image scales as the spectral features are not expected to be scale dependent. 478

479 In general images produced with the Helikite were of reasonable quality, but only the images taken from the tripod and the bridge were consistently of good quality. In particular collection of NIR 480 photos from the more elevated platforms was difficult due to limited availability of the camera 481 autofocus in combination with the light blocking filters. The NIR(R72) band of the image taken from 482 the pole was especially blurry, which may have affected some of the results. Texture features are 483 likely to be more dependent on image focus than shape features like the l/w ratio, but further 484 investigations of such effects is required. No pre-processing was applied to any of the data. Some 485 preprocessing could have further improved data quality, as sunglint caused locally high reflectance 486 values in most image bands. The high values will have affected contrast calculations, resulting in an 487 overestimation of object contrast. Its quantitative effect on the presented results is currently not 488 known. 489

490 The first attempt to use an UAS to collect remote sensing data for submerged macrophyte monitoring was not overly successful. This was to a large extent due to the type of UAS and multi-491 spectral sensor used. Due to a combination of camera weight, wind conditions, presence of 492 493 surrounding vegetation, people and telegraph lines it was impossible to achieve elevations higher than the telescopic pole with the Helikite and therefore scale wise this platform did not contribute extra 494 495 information to this study and the range of scales studied was limited. Because the exact location of the camera from this platform was most difficult to control, only a very small section of the images was 496 ultimately suitable for analysis. It also made manual image correction rather challenging. The Helikite 497 498 required restricted environmental conditions, especially when paired with a relatively heavy camera.

Similar to the spectral discrimination analysis, the object-based features may 'interact' and perform better when a number of different features are combined to discriminate between plant species. This has currently not been attempted yet. So far the difference tests are statistical exercise only. Better results are also likely with the inclusion of band ratios. To find out to what extent the features really enable accurate classification of the macrophyte species will need further testing on more extensive image data, covering larger areas and a wider range of situations.

505 Implications

506 The foregoing discussion suggests that it is not possible to accurately map submerged aquatic vegetation in the chalk streams, using spectral information only, even if water depth correction of the 507 508 vegetation spectra is possible. However, despite strongly increasing K_d the NIR wavelengths still show considerable amounts of reflectance at the submergence depths observed for the chalks stream 509 macrophyte species. The observation that wavelengths between 920 and 950nm showed potential to 510 discriminate between at least two of the submerged species, was remarkable and use of this 511 wavelength region should be further explored. Although spectral separability in the NIR wavelengths 512 513 was not clearly confirmed by J-M distance analysis, the information in any case enhances shape and 514 textural variation in the data, which benefits the OBIA approach. The inclusion of texture and shape features in image analysis through OBIA clearly shows promise for the mapping of SAV from image 515 data. Further work is however also required on scale dependency, as shape and texture features did 516 517 not show significant differences between the species for all scale levels.

Finally, to make the presented techniques interesting for river managers for mapping and monitoring of SAV patterns in small streams the proposed approach will need to be converted into a tool that can produce consistent results for a wide range of fluvial situations with the smallest amount of input from operators. The OBIA approach has already shown to be a useful approach in other settings, by eliminating the need for an image data sample for classification after a rule set has been created (e.g. Walker and Blaschke, 2008). Based on the results of this study it is not inconceivable that a similar tool can be developed for the benefit of shallow clear stream environments.

525

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618 Figure captions

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Figure 1: The transmission spectra of BP1 bandpass and CC1 and R72 blocking filters based on
 manufacturers specifications (maxmax.com). Submerged macrophyte spectrum included with dashed
 line for illustration.

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Figure 2: Close-up of sub-objects in 'from tripod' image. Left: Pondweed objects. Right: moreelongated Water Crowfoot objects.

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Figure 3: Attenuation of reflectance from Water Crowfoot for submergence depths between 1.5 and40cm (example based on data collected during this study).

629630 Figure 4: Frequency of statistically significant differences between three submerged macrophyte

species with ANOVA analysis. Bars show number of significantly different combinations obtained
 (dark grey 99%; light grey 95%). Spectra represent min, max and average signatures for each of the
 species.

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Figure 5: Frequency of statistically significant differences for three out-of-the-water macrophyte species with ANOVA analysis. Bars show number of significantly different combinations (dark grey

637 99% significant; light grey 95% significant).

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Figures 6 A-D: A selection of image 'bands' with segmentation object outlines: A) from tripod
NIR(R72) band; B) from bridge Green band; C) from pole NIR(BP1) band; D) from helikite
NIR(R72) band. In all images white object outlines represent Pondweed, grey Water Crowfoot and
black Unclassified.

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Figure 7: Boxplots illustrating the object feature values for 8 features (A-H), comparing Pondweed and Water Crowfoot objects as derived from images taken from four different platforms.

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TABLES

651 Table 1: Type and number of submerged vegetation spectral samples

Macrophyte species	Ν	Depth range (cm)
Pondweed	60	10-25
Water Crowfoot	37	2-40
Water Milfoil	66	3-50

Table 2: Results of the Jeffries-Matusita distance analysis for combinations of submerged Pondweed,
Water Crowfoot and Spiked Water Milfoil. White font indicates highest achieved distance value for
discriminating a pair of species; intermediate grey shade indicates highest distance value for given
band combination; light grey second best.

No.	Band selection method	Wavelengths (nm)	J-M distance value for species combination			
Bands			Pondweed - Milfoil	Crowfoot - Milfoil	Pondweed - Crowfoot	
2	Random from significant wavelengths	555; 935	1.08	0.61	0.40	
	Random from all wavelengths	1054; 426	0.54	0.10	0.30	
		609; 816	0.67	0.45	0.09	
3	Random from significant wavelengths	935; 544; 555	1.40	0.82	0.75	
	Random from all wavelengths	848; 539; 489	1.45	1.21	0.34	
	-	912; 756; 884	1.08	0.61	0.48	
4	CART with all wavelengths	545; 576; 966; 555	1.54	1.31	0.66	
	CART with all wavelengths; No Milfoil	533; 689; 997; 550	1.61	1.18	0.85	
	Random from significant wavelengths	577; 927; 919; 568	1.69	1.37	0.88	
	-	553; 923; 914; 525	1.72	1.15	1.14	
	Random from all wavelengths	868; 522; 892; 1011	0.97	0.58	0.42	
	-	830; 639; 813; 773	1.08	0.96	0.28	
5	CART with significant wavelengths	543; 926; 555; 537; 923	1.72	1.20	1.26	
	Random from significant wavelengths	535; 925; 914; 554; 540	1.78	1.40	1.25	
		562; 925; 540; 891; 573	1.72	1.49	0.87	
	Random from all wavelengths	935; 559; 939; 544; 555	1.77	1.51	1.10	
		469; 911; 562; 514; 703	1.81	1.33	1.62	
6	CART with significant wavelengths; No Milfoil	533; 525; 923; 553; 913; 917	1.80	1.53	1.27	
	Random from significant wavelengths	535; 919; 548; 575; 924; 561	1.87	1.60	1.45	
	Random from all wavelengths	920; 566; 925; 914; 554; 540	1.84	1.66	1.40	

Table 3: Results of the Jeffries-Matusita distance analysis for combinations of Pondweed, Water Crowfoot and Spiked Water Milfoil measured on canvas sheet. White font indicates highest achieved

distance value for discriminating a pair of species; intermediate grey shade indicates highest distance

No. Bands	Band selection method	Wavelengths (nm)	J-M distance value for species combination			
			Pondweed -	Crowfoot -	Pondweed -	
			Milfoil	Milfoil	Crowfoot	
3	CART with all wavelengths	711; 465; 483	1.84	1.69	0.73	
	CART with all wavelengths; No Milfoil	465; 535; 483	2.00	1.93	0.77	
4	Random from all wavelengths	545; 576; 966; 555	2.00	1.99	1.18	
	Random from significant wavelengths	710; 752; 522; 834	2.00	1.98	1.26	
5	Random from all wavelengths	543; 926; 555; 537; 923	2.00	1.98	1.44	

value for given band combination; light grey second best.

667 Table 4. Object sample numbers N for each macrophyte and location.

	N Pond-	N Water
	weed	Crowfoot
From tripod	12	21
From bridge	2	14
From helikite	3	8
From pole	6	9

Table 5. Results for the Mann-Whitney U non-parametric test of similarity. Shaded results are

significant at 95%.

I	Test statistic						
Location	Signific ance	Mean R	Mean G	Mean B	Mean BP1	Mean R72	Mean R72+BP1
from tripod	U	108	57	68	131	113	121
(1.5m)	р	0.50	0.00	0.02	0.68	0.41	0.75
from bridge	U	7	7	9	6	8	5
(3m)	р	0.33	0.33	0.50	0.27	0.42	0.20
from helikite	U	3	7	9		0	
(~5m)	р	0.12	0.38	0.83		0.02	
from pole	U	20	16	9	15	10	16
(5.4m)	р	0.46	0.22	0.04	0.18	0.05	0.22

Location	Test statistic	Mean Length/Wi	Mean Stdev Red		GLCM Contrast (quick	GLCM Dissimilari ty (quick	GLCM Homogene ity (quick
	Signific	dth sub-	Sub-		8/11) (all	8/11) (all	8/11) (all
	ance	objects	objects	Stdev Red	dır.)	dır.)	dır.)
from tripod	U	16	30	46	25	27	33
(1.5m)	р	0.00	0.00	0.00	0.01	0.00	0.00
from bridge	U	1	4	7	4	6	7
(3m)	р	0.03	0.15	0.33	0.15	0.27	0.33
from helikite	U	0	2	7	2	2	2
(~5m)	р	0.02	0.07	0.37	0.07	0.07	0.07
from pole	U	22	8	11	1	3	3
(5.4m)	р	0.61	0.03	0.07	0.00	0.00	0.00

Figures





680 Figure 1





683 Figure 2







689 Figure 4





695 Figure 6



698 Figure 7