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Measuring submerged macrophyte standing crop in shallow rivers: a test of methodology.

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Abstract

High natural variability in space and time can make accurate measurements of macrophyte standing crop difficult. Accuracy of such measurements could be improved quantifying the relationships between the different methods of measuring standing crop which are available to researchers. In this study we compare cover, volume, and biomass as measures of standing crop. Percentage cover, percentage volume, and dry weight biomass estimates were positively related ($R^2_{(adj)}$ range = 54 – 96 %), but these relationships were significantly different between sites, and to a lesser extent between months. Biomass was related ($R^2_{(adj)}$ range = 18 – 73 %) to stand height. Furthermore, cover, volume and biomass indicated different seasonal trends in standing crop at the two study sites. Our study presents a suite of standing crop measures that exhibit close congruence, can be measured efficiently and minimise destructive sampling *in situ*, attributes which will aid in the design and implementation of future macrophyte measurement protocols for shallow rivers.

1. Introduction

A wide range of studies have documented the important roles macrophytes play in the structure and functioning of aquatic ecosystems (i.e. Landers, 1982; Carpenter and Lodge, 1986; Cotton et al., 2006). Quantitative assessments of these roles require accurate measurements of standing crop. However, plant standing crop varies in both space and time, and measurements can be costly in terms of time and effort, highlighting the need to develop efficient sampling methods (Downing and Anderson, 1985; Spears et al., 2009; Gunn et al., 2010). The three most commonly used measures of standing crop are (a) cover (%), the proportion of a given benthic area occupied by macrophytes, (b) volume occupied (%), the proportion of a given volume of water occupied by macrophytes, and (c) biomass (g m^{-2}), the mass of plant material in a given area or volume (Murphy, 1990; Gunn et al., 2010). Traditional methods of biomass estimation are destructive (e.g. Hiley et al., 1981; O'Hare et al., 2010a; Johnson and Newman, 2011), which in many instances is not desirable; for example, when removal of biomass interferes with subsequent observations, in studies of protected species, or in the study of animals associated with macrophytes where removal of biomass may alter the behaviour or abundance of the study animal (Gourard et al., 2008; Wood et al., 2012; Wood et al. in press). There is therefore a need to develop and test methods that either remove or translocate the destruction outside the area of interest, whilst retaining the ability to accurately estimate biomass. Alternatively, the ability to use cover or volume as a robust surrogate for biomass would remove the need for destructive biomass sampling.

Whilst cover, biomass and volume have been used interchangeably as measures of standing crop, they may not concur as each represents a different aspect of the macrophyte stand. There is some limited evidence to suggest a positive relationship between cover and biomass (Dawson, 1978; O'Hare et al., 2010b; Yin et al., 2011). However, due to the spatiotemporal variability of plant stand structure, the strength of such relationships may not be constant across space and time (Downing and Anderson, 1985). Given the importance of accurate estimates of plant standing crop for conservation, hydrological and environmental management purposes, there is a need to (i) quantify the relationships between the cover, volume and biomass of aquatic plant stands, and (i) determine how such relationships vary in space and time.

This study tested two hypotheses regarding the relationships between plant cover, biomass and volume. The first hypothesis was that these three measures of plant standing crop would be positively related. The second hypothesis was that these relationships would vary in space (i.e. between sites) and time (i.e. between months).

2. Methods

2.1 Study sites

This study was conducted between March and September 2009, covering the macrophyte growth cycle from growth to recession (Ham et al., 1981; Flynn et al., 2002; Cotton et al., 2006), at two sites on a mesotrophic chalk river, the River Frome (Dorset, UK); a headwater site at Maiden Newton and a mid-reaches site at East Stoke (Table 1). Detailed site information can be found in Wharton et al. (2006). The River Frome macrophyte assemblage is almost exclusively dominated by *Ranunculus penicillatus ssp. pseudofluitans* (Syne) S.D. Webster, with *Potamogeton spp.*, *Elodea canadensis* Michx., *Zannichellia palustris* L., *Sparganium emersum* Rehmman, *Oenanthe fluviatilis* (Bab.) Coleman, *Nasturtium officinale* W.T. Aiton, and *Myriophyllum spicatum* L., also present in low abundance at East Stoke (O'Hare et al., 2007; Wood et al., 2012).

2.2 Measuring macrophyte cover, volume, and stand height

At each site a 10 m-long reach, characteristic of that site, was selected from which measurements were made in March, May, July and September. Before any in-stream measurements were made, macrophyte cover was estimated visually from the bank-side in 5 % increments at the upstream limit of the reach. For the in-stream estimates of cover, we began at the bottom of the 10 m reach and walked upstream. At 1 m intervals, transects were measured across the entire width of river, with a 0.5 x 0.5 m quadrat laid end-on-end. For each quadrat, the percentage cover of each macrophyte species was estimated in 5 % increments. Depth was measured at the quadrat centre to the nearest 0.05 m and the quadrat volume (m^3) was calculated as the quadrat area (0.25 m^2) multiplied by depth (m). If a macrophyte stand was present within the quadrat, depths to the top and bottom of the plant nearest to the centre of the quadrat were measured to the nearest 0.05 m. Macrophyte stand height (m) was given by subtracting these depths from total depth. Macrophyte volume (m^3) was calculated from the area covered by macrophytes (m^2) multiplied by the stand height (m). The percentage of quadrat volume occupied by macrophytes was calculated as (macrophyte volume / quadrat volume) * 100. The same quadrats were measured in March, May, July and September.

2.3 Estimating biomass from stand height

Biomass samples were taken with a 0.00785 m² hand corer immediately downstream of each 10 m reach where cover and volume had been measured. Thirty samples were taken each month at different downstream locations on each sampling occasion to preclude the effects of previous biomass removal, but all samples were taken within 40 m of the original study reach, in reaches with similar morphological and habitat characteristics. Samples were taken at each site in March, May, July and September, each from a different *R. pseudofluitans* plant. *R. pseudofluitans* was selected as it dominates the chalk river macrophyte assemblage and our study reaches (Dawson, 1976; Flynn et al., 2002; Wood et al., 2012). Before each sample was taken the water depth, and depths to the top and bottom of the plant were recorded (± 0.05 m). The sampler then placed their hand underneath the plant stand and lowered the corer onto the hand, trapping part of the stand in the corer (Westlake et al., 1986). The plant material outside of the corer was then trimmed off and the sample labelled. In the laboratory non- *R. pseudofluitans* material was carefully removed and the *R. pseudofluitans* sample dried to constant weight at 60 °C using a Heraeus Kelvitron T oven (Thermo Fisher Scientific, Loughborough, UK). Dry mass was measured to within ± 0.01 g on a Sartorius PT120 balance (Sartorius GMBH, Germany).

2.4 Statistical analyses

All statistical analyses were carried out using SPSS version 18 (IBM, US), with a statistically significant result attributed where $p < 0.05$. Normality of the regression and model residuals was confirmed for all data. To examine the consistency between sites and between months of the relationship between the values per quadrat for percentage cover (C), volume occupied (V), and dry weight biomass (B) we tested mixed effect repeated measures models (using SPSS routine MIXED) of (i) cover (%) with volume (%) as a covariate, (ii) cover (%) with biomass (g m⁻²) as a covariate, and (iii) volume (%) with biomass (g m⁻²) as a covariate; in each model, site and month were fixed factors and sampling quadrat within site was treated as a random factor subject to repeated measurement (March, May, July, September) with auto-regressive AR(1) auto-correlations between successive sampling months. For each site-month combination a linear regression analysis was used to assess the relationship between the three measures of standing crop. Linear regression was also used to compare percentage cover estimated visually from the riverbank and mean measured cover values using in-stream quadrats for each reach for all months. To examine the consistency between sites and between months of the relationship between the biomass and height of macrophyte stands we tested a univariate GLM of log₁₀-

transformed R_{CI} (range of the confidence intervals) with \log_{10} -transformed sample size as a covariate and site and month as fixed factors. As different macrophyte stands were sampled on each occasion, a repeated measures analysis was not appropriate here. To calculate the biomass per reach per month at each site, the height-mass regression relationship derived downstream of the reach was applied to the measured stand height values within the reach to give the mass per hypothetical core for each quadrat. This value was divided by the core area (0.00785 m^2) and then multiplied by the area covered by macrophytes in that quadrat (m^2) to yield the biomass. As the quadrat area was 0.25 m^2 , the biomass per square metre for each quadrat was calculated by multiplying the quadrat biomass by 4. Total biomass per 10 m reach was calculated as the average biomass per quadrat (m^{-2}) multiplied by the reach area (m^2).

3. Results

3.1 Cover, volume, and biomass measures

At Maiden Newton, macrophyte percentage cover increased from March to a maximum in July and decreased thereafter, whilst at East Stoke cover increased consistently from March to September (Fig. 1a). Small-scale variation (i.e. between 0.25 m^2 quadrats) was high in all months at each site (Fig. 2). The macrophyte assemblage was dominated by *R. pseudofluitans* in all months, comprising a mean ($\pm 95 \%$ CI) of $99 \pm 1 \%$ of the total macrophyte cover at Maiden Newton and $92 \pm 3 \%$ at East Stoke. The remainder of plant cover was comprised of limited quantities of *Potamogeton spp.*, *E. canadensis*, *Z. palustris*, *S. emersum*, *Oenanthe spp.*, *N. officinale*, and *M. spicatum* at East Stoke, and *Oenanthe spp.* at Maiden Newton.

At Maiden Newton, percentage volume occupied by macrophytes (mean $\pm 95 \%$ confidence interval) followed a similar increase from March as cover ($13.6 \pm 1.2 \%$, $n = 102$) but peaked sooner in May ($34.2 \pm 6.4 \%$, $n = 104$) before decreasing thereafter. At East Stoke, volume mirrored the patterns observed for cover by increasing consistently from March (6.9% , $\pm 1.2 \%$, $n = 309$) to September (22.1% $\pm 2.1 \%$, $n = 302$) (Fig. 1c). In the mixed effects repeated measures models allowing for the potential of interaction between both site and month with the slope of the relationship with the covariate, all interactions with covariate slope were statistically significant for the cover versus volume, cover versus biomass and volume versus biomass relationships (Table 2). Thus we carried out linear regression analyses between cover, volume, and biomass for each site-month combination; cover, volume, and biomass were strongly, positively related in all months at both sites (Table 3).

A significant relationship was also detected between in-stream and bank-side estimates of cover ($F_{1,6} = 12.01$, $p = 0.0132$, $R^2_{(adj)} = 61.3\%$; Fig. 3), over the range of measured cover values tested (24.9 – 68.2 %).

3.2 Biomass measures and estimates from stand height

Macrophyte biomass (mean \pm 95 % confidence interval) at Maiden Newton increased from 161.7 ± 50.1 g dry Wt m^{-2} ($n = 102$) in March to 398.4 ± 74.5 , g dry Wt m^{-2} ($n = 91$) in July, before declining to 169.4 ± 49.8 g dry Wt m^{-2} ($n = 94$) in September, while at East Stoke biomass increased from 74.0 ± 12.2 %, g dry Wt m^{-2} ($n = 309$) in March to 222.9 ± 22.5 g dry Wt m^{-2} ($n = 302$) in September (Fig. 1b). However, the East Stoke reach had a consistently greater total dry Wt biomass per 10 m reach due to the greater river width (15.5 m versus 5.1 m; Table 1); total biomass per 10 m reach at Maiden Newton increased from 8246.9 g in March to 20318.4 g in July, before declining to 8641.0 g in September. At East Stoke total reach biomass increased consistently from 11465.5 g in March to 34550.9 g in September. A GLM of macrophyte biomass-stand height relationships indicated that a model in which intercept and slope were dependent on both site and month best explained the variance in biomass ($F_{9,230} = 46.039$, $p < 0.001$, $R^2_{(adj)} = 62.9\%$). Therefore site- and month-specific height-mass equations were used to calculate biomass in the 10 m reaches (Table 4).

4. Discussion

In this study we have quantified the relationships between the three most commonly-used measures of plant standing crop; cover, volume and biomass. We are not aware of any previous study that has examined these relationships. Strong, positive relationships were detected between all three measures, but such relationships typically varied between site and month. We demonstrated the application of a method of biomass measurement which avoided influencing subsequent macrophyte growth and biomass by relocating destructive sampling downstream of the main study reach. The results of both analyses will facilitate efficient future sampling in shallow rivers.

Our estimated values of macrophyte standing crop over a cycle of growth and recession were within the seasonal ranges reported in other studies of the chalk river macrophyte community (Dawson, 1976; Ham et al., 1981; Armitage and Cannan, 2000; Flynn et al., 2002). Percentage cover, percentage volume, and dry weight

biomass co-varied in accordance with our first hypothesis, concordant with previous studies which had reported positive relationships (O'Hare et al., 2010b; Yin et al., 2011). However, a 1:1 relationship was not found for any relationship. The relationships between cover and biomass or volume are likely to reflect the trade-off between the horizontal and vertical growth of plant stands (Duartes et al., 1996). In particular percentage volume values were always lower than percentage cover, probably reflecting the growth form of *R. pseudofluitans* which often maximises canopy at the water surface (Dawson and Robinson, 1984). Macrophytes such as *R. pseudofluitans*, which spread at the surface over large areas from rooted stems, will typically have cover values which are high relative to biomass or volume (Edwards and Brown, 1960). Thus plant morphology and growth form are likely to influence the relationships between cover, biomass and volume. As volume also indicated a slightly different seasonal trend in standing crop than cover or biomass, cross comparisons of data derived by the different methods should be treated cautiously and attention paid to the growth form of the study species. The stems produced by *R. pseudofluitans* during the flowering phase, April to June, are more buoyant than stems produced at other times of year, which could result in a higher volume per cover/biomass during the flowering phase (Dawson, 1976). However, we found no evidence that the relationships between volume and cover or biomass became biased towards volume during the flowering phase; thus the increase in stand volume observed in May likely reflected an increase in the quantity of plant material both horizontally and vertically in the water column, hence the observed concomitant increases in cover and biomass. In addition, the relationships between cover, volume and biomass varied in space (i.e. between sites) and time (i.e. between months) in accordance with our second hypothesis. Such variance may be due, at least in part, to differences in plant morphology; the architectural properties of aquatic plants (i.e. leaf size) are known to vary within-species with differences in both season and habitat (Duartes, 1991). Species other than *R. pseudofluitans* were of minor importance (< 8 % total cover). Thus it is unlikely that spatiotemporal variance in the relatedness of measures of standing crop was influenced by changes in plant community composition or the relative importance of different species with contrasting architectures.

The use of sampling methods that measure biomass indirectly by relocating destruction outside the main study area can facilitate the temporal study of protected species and animals associated with macrophytes where removal of biomass may alter the behaviour or abundance of the study animal (Gourard et al., 2008; Wood et al., 2012; Wood et al. in press), where it is critical that the sampling method does not modify the existing biomass or its growth. However, a potential source of error in translocating destructive sampling to a secondary

area is that differences in the stand height-biomass relationship may exist between the two areas. Indeed in this study we detected that the stand height-biomass relationship differed between sites, although substantial physical and hydrological differences existed between our sites (Table 1). Future studies using this method could minimise potential error due to between-site differences by adopting our approach of translocating the destructive sampling over the shortest possible distance. A full validation would test the method against destructive *in situ* sampling in synchrony in the same area. However, destructive sampling is only likely to bias such studies if sampling removes or impacts on the growth of a sufficient quantity of macrophyte material to influence subsequent measures of abundance. In this study the removed material as a proportion of the macrophyte biomass in a reach was negligible; even where macrophyte biomass was lowest (Maiden Newton, March) sampling-related destruction accounted for < 1.4 % of biomass within a 10 m reach. Wright et al. (1981) concluded that repeated monthly sampling of chalk river macrophytes (area = 0.05 m², *n* = 25 per month), using a similar method to this study, did not affect subsequent measures of abundance. Therefore, repeated sampling may not affect future macrophyte abundance, when both the sampler size and sample number are small. In plant habitats where these assumptions are met, indirect biomass estimation could provide a useful means of obtaining accurate estimates of biomass without destructively sampling in the immediate areas of interest.

Comparison of cover estimates made in-stream and on the bank-side suggested that visually estimating macrophyte cover from the river bank resulted in an overestimate of cover (127 %) relative to in-stream estimates. Whether the consistent overestimation of cover is an inherent bias in the visual estimate method per se or an individual bias is impossible to determine as only one observer was tested in our study. Similarly the low spatial replication (*n* = 2) means that we were unable to test whether overestimation was more likely at sites with certain hydrogeomorphological characteristics. An advantage of visual estimates of cover is that they take much less time than in-stream measurements, and thus reduce required sampling effort and cost. In this study visual estimation took less than one minute at each site, compared with the 120 (Maiden Newton) and 240 (East Stoke) minutes required for in-stream measurements. Our results suggest that as a way of measuring macrophyte cover, bank estimates can be a much quicker, if less accurate method than in-stream measurements. Our results are reassuring given the widespread use of bank-based cover estimates in routine monitoring programmes, such as the Mean Trophic Rank Methodology which is based on rapidly assessing a 100 m reach (Dawson et al., 1999).

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Tables

Table 1: Characteristics of the two River Frome study sites over the March – September study period. ^a data (1998-2003) from Wharton et al. (2006).

	Maiden Newton	East Stoke
Latitude, Longitude	50°46'N, 02°34'W	50°41'N, 02°11'W
Length of study reach (m)	10.0	10.0
Mean channel width (m)	5.1	15.5
Mean depth (m)	0.33	0.45
Area of study reach (m ²)	51.0	155.0
Riparian shading (%)	10	0
Mean Q (m ³ s ⁻¹)	0.6 ^a	5.5 ^a
Peak Q (m ³ s ⁻¹)	1.6 ^a	24.0 ^a

Table 2: Mixed model significance test p value for each factor (Site, Month and Covariate) and their interactions, together with the estimation temporal auto-correlation (AR(1)) of the repeat bi-monthly measurements on each sample quadrat of each site.

Mixed Model term	Y variable ~ Covariate		
	Cover ~Volume	Cover ~ Biomass	Volume ~ Biomass
-			
Site	0.386	0.725	0.003
Month	<0.001	<0.001	0.108
Site x Month	<0.001	<0.001	0.336
Covariate	<0.001	<0.001	<0.001
Site x Covariate	<0.001	<0.001	0.003
Month x Covariate	<0.001	<0.001	<0.001
Site x Month x Covariate	<0.001	<0.001	<0.001
Auto-Correlation AR(1)	0.043	0.083	0.174

Table 3: The results of the linear regression relationships between macrophyte cover (%), percentage volume occupied (%), and dry weight biomass (g m^{-2}) for each month at the two sites. All relationships were significant ($p < 0.0001$).

Comparison	Site	Month	Intercept (\pm SE)	Slope (\pm SE)	d.f.	R^2 (adj)
Cover-Volume	Maiden Newton	March	6.47 ± 1.53	1.36 ± 0.06	101	85 %
		May	17.03 ± 2.94	1.12 ± 0.06	103	76 %
		July	28.17 ± 3.52	1.08 ± 0.09	90	60 %
		Sept.	19.10 ± 2.63	1.09 ± 0.07	93	69 %
	East Stoke	March	9.48 ± 1.12	2.66 ± 0.09	308	75 %
		May	12.62 ± 1.29	2.18 ± 0.08	314	71 %
		July	14.49 ± 1.37	2.62 ± 0.08	318	79 %
		Sept.	35.10 ± 2.09	1.50 ± 0.07	301	59 %
Cover-Biomass	Maiden Newton	March	-12.33 ± 13.13	6.99 ± 0.31	101	83 %
		May	-5.48 ± 8.16	1.72 ± 0.12	103	68 %
		July	-54.65 ± 36.53	8.02 ± 0.54	90	71 %
		Sept.	-42.00 ± 22.35	5.20 ± 0.40	93	64 %
	East Stoke	March	-6.88 ± 4.05	2.90 ± 0.09	308	75 %
		May	-15.32 ± 8.27	4.32 ± 0.17	314	67 %
		July	-19.38 ± 8.09	3.84 ± 0.13	318	73 %
		Sept.	-50.57 ± 16.42	4.01 ± 0.21	301	54 %
Volume-Biomass	Maiden Newton	March	22.25 ± 12.53	10.27 ± 0.47	101	82 %
		May	8.40 ± 5.80	2.38 ± 0.12	103	79 %
		July	114.82 ± 30.29	10.80 ± 0.80	90	67 %
		Sept.	25.60 ± 15.75	7.28 ± 0.45	93	74 %
	East Stoke	March	4.33 ± 1.52	10.06 ± 0.12	308	96 %
		May	3.79 ± 3.52	13.08 ± 0.21	314	92 %
		July	1.77 ± 3.79	12.76 ± 0.21	318	92 %
		Sept.	-1.00 ± 5.26	10.13 ± 0.18	301	91 %

Table 4: The results of the linear regression relationships between dry biomass per core (g) and macrophyte stand height (m) for each month the two sites. All relationships were significant ($p < 0.01$).

Site	Month	Intercept (\pm SE)	Slope (\pm SE)	d.f.	$R^2_{(adj)}$
Maiden Newton	March	0.93 ± 0.56	21.20 ± 3.55	29	54
	May	1.21 ± 0.54	20.64 ± 3.09	29	60
	July	-1.66 ± 1.28	60.61 ± 9.69	29	57
	Sept.	0.83 ± 0.70	25.53 ± 4.37	29	53
East Stoke	March	-0.42 ± 0.25	22.65 ± 2.53	29	73
	May	1.97 ± 0.48	11.91 ± 4.39	29	18
	July	-0.09 ± 0.45	25.93 ± 5.09	29	46
	Sept.	0.28 ± 0.47	14.44 ± 2.35	29	56

Figures

Figure 1: Seasonal changes in mean (\pm 95 % confidence intervals) macrophyte cover (a), dry biomass (b), and volume (c) at Maiden Newton (MN) and East Stoke (ES).

Figure 2: Block diagrams indicating the variability in percentage macrophyte cover in 0.5 x 0.5 m quadrats in a 10 x 5 m section of river at the two sites. The river flowed right to left.

Figure 3: The linear relationship (\pm SE) between in-stream and bank-side estimates of percentage macrophyte cover, based on mean data for all sites and months. A 1:1 line is included for comparison.

Figure 1

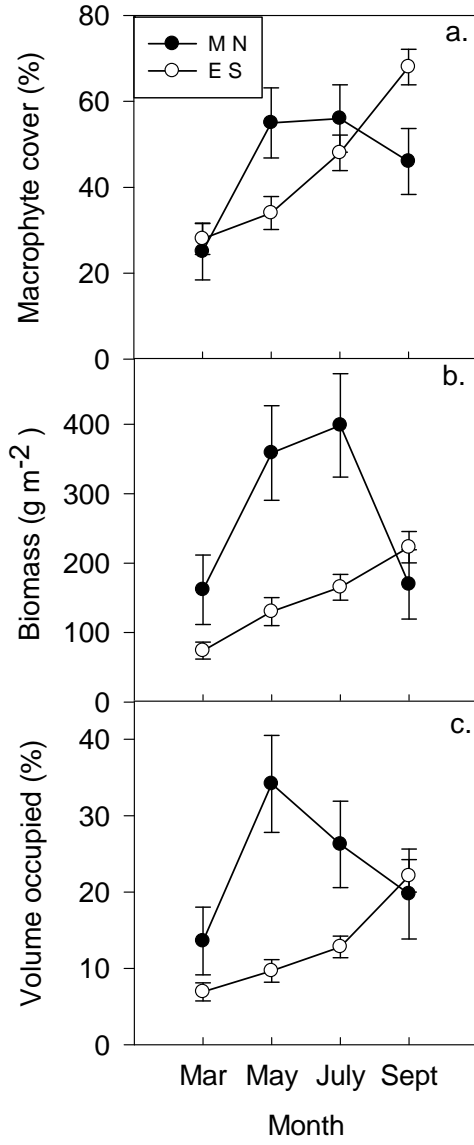


Figure 2:

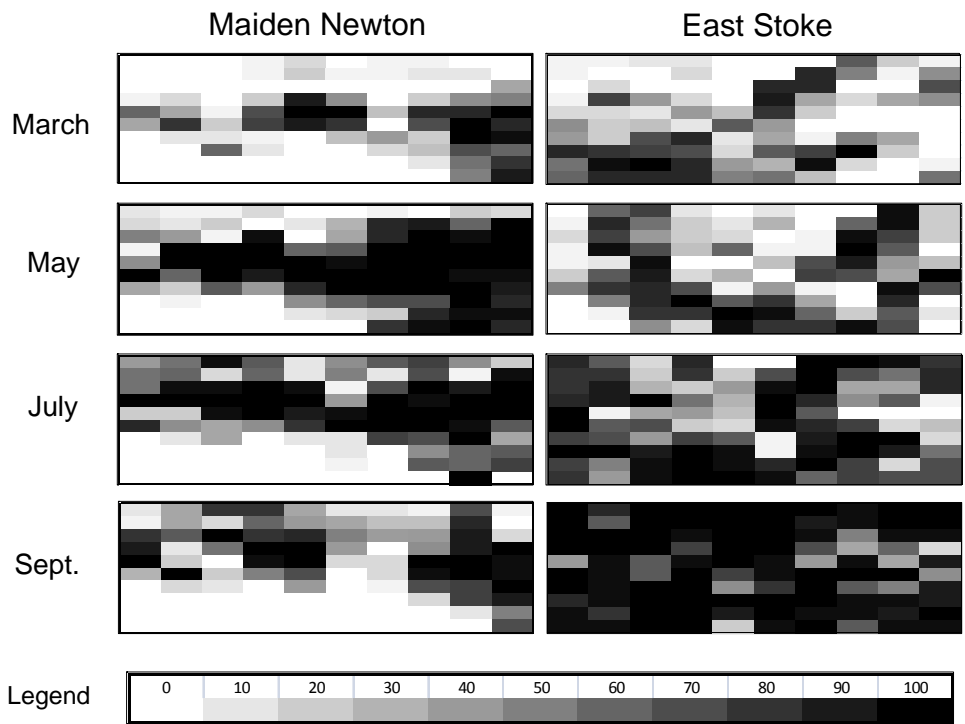


Figure 3:

