Measuring shoot length of submerged aquatic plants using graph analysis

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ABSTRACT

The fate of herbicides in experimental studies can be observed by measuring the total shoot lenght of submerged aquatic plants, such as *M. spicatum* and *E. canadensis*. Manual measurement of the shoot lengths takes a lot of time and is difficult to trace. An ImageJ application has been developed to measure the individual shoot length of the plants taken by a digital picture. After thresholding the picture, the binary image is transferred to a skeleton and the skeleton to a weighted graph using the ImageJ plug-in Analyze-Skeleton. The nodes in the graph are the junction pixels and the edges are the slab pixels. The weight of the edges corresponds with their length. In computer science, the FloydWarshall algorithm is a graph analysis algorithm for finding shortest paths in a weighted graph. A single execution of the algorithm will find the lengths (summed weights) of the shortest paths between all pairs of nodes. The longest length can be considered as a good estimation of the total length of the plant. The warshall algorithm was implemented in the Analyze-Skeleton plugin and used to measure the length of a large number of plants of both specimens. The lengths measured this way were compared with manual measurements. The R^2 was 0.94 for *E. canadensis* and 0.91 for *M. spicatum*. From those high R^2 values we can conclude that the algorithm works very well.

Keywords: plants, shoot length, skeleton, pruning, nodes, edges, Floyd-Warshall, shortest path.

1. INTRODUCTION

In aquatic risk assessment procedures for herbicides within the context of EU Regulation 1109/2009/EC, tests with rooted submerged macrophytes may be required. The ecotoxicological impact of herbicides in experimental studies can be observed by measuring concentration-response relationships for the endpoint total shoot length of submerged aquatic plants, such as *Myriophyllum spicatum L.* (Eurasian Watermilfoil) (Figure 1(a)) and *Elodea canadensis* (American Waterweed)(Figure 1(b)), as recommended by the HARAP workshop.[1]

Traditionally these lengths are measured manually, but this takes a lot of time and is difficult to trace. Automatic measurements using a digital photograph and ImageJ was researched. In first instance after thresholding the image lengths were measured with standard measures from 'Analyze Particles'. Unfortunately none of those measures gave good correlation with the manual measures. Therefore a new approach was developed using the skeleton of the binary objects.

2. EXPERIMENTAL SETUP

After harvesting, the plants are cut in pieces and placed on a back illumination table. In order to be able to measure the plant color accurately also front illumination was used. The object was coded with a QR-Code placed on the table and a calibration disk with a fixed size of 50 mm diameter (figure 1). Images are recorded with a Nikon D90 Camera equipped with a Nikon AF-S DX NIKKOR 16-85mm objective lens. Before recording the images the total shoot length was measured manually. One to three images were needed per plant to hold all the pieces. The images were analyzed using the procedure described in section 3. Finally the manual and automated measurements were compared.

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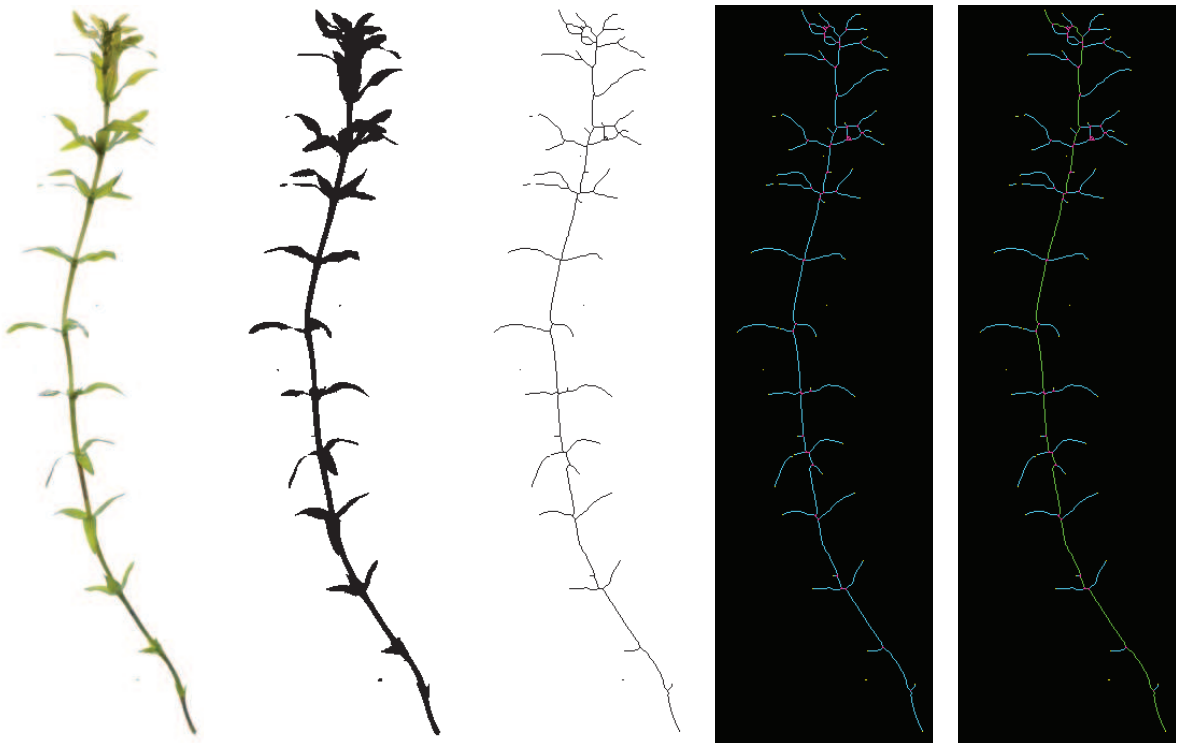
(a) *M. spicatum*

(b) *E. canadensis*

Figure 1. Example image of *M. spicatum* (a) and *E. canadensis* (b). The upper-left corners shows the QR-Coded object information along with a calibration disk.



(a) Color (b) Binary (c) Skeleton (d) Tagged (e) Longest shortest path

Figure 2. Processing steps for a piece of *E. canadensis*. An color image (a) is thresholded to a binary image (b). From the binary image a skeleton (c) is calculated. The skeleton is tagged (d) and the longest shortest path (e) is calculated.

3. PROCEDURE

3.1 Skeleton

In binary image processing repeatably removing pixels from the edges of objects, while keeping the connectivity of the object intact, results in single pixel wide skeletons. These skeletons can be used to measure shape parameters. Van Eck *et. al.* (1998)[2] used the skeleton for measuring the length of cucumber fruits. This was easily accomplished since cucumber fruits are smooth objects and the constructed skeleton did not have any side branches. In our case the plants have lots of leaves and side branches which results in a very complicated skeleton. Figure 2 shows the steps which were carried out on a small example image of *E. canadensis*. First a fixed threshold was applied on the color image 2(a) resulting in a binary image (2(b)). Figure 2(c) shows the skeleton of the binary image. Thresholding and skeletonizing were applied using standard ImageJ functions.

Using the Analyze Skeleton plugin[3] the skeleton is annotated. This plugin tags all pixels in the skeleton image and then counts all its junctions, triple and quadruple points and branches, and measures their average and maximum length. Although the plugin is suitable to annotate 3D skeletons (26-connected pixels) in our case it is used in 2D only.

The pixels are classified into three different categories depending on their 8 neighbors:

- End-point pixels: if they have less than 2 neighbors.
- Junction pixels: if they have more than 2 neighbors.
- Slab pixels: if they have exactly 2 neighbors.

Figure 3. Tagged skeleton, zoomed into top portion of figure 2(e).

Figure 2(d) shows the result. The end-point pixels are colored yellow, the junction pixels purple and the slab pixels cyan.

3.2 Longest shortest path

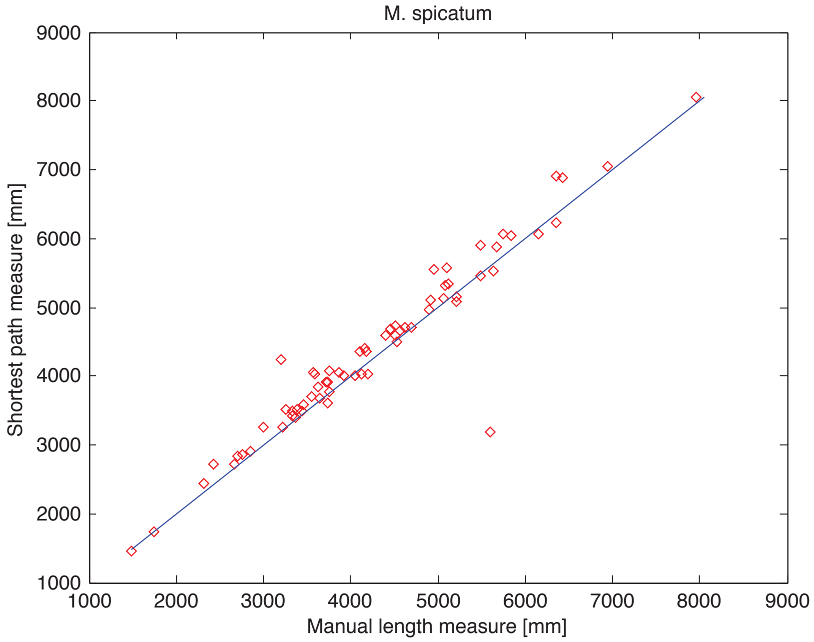
The tagged skeleton can be described as an undirected and weighted graph. The nodes are the end-point and junction pixels, the weighted edges are the summed euclidian distances between every slab pixel and its neighbor in the edge. From graph theory it is known that there always is a shortest path between two nodes of a connected graph. So, in a graph there always exists a longest, shortest path, which is the best approximation for the plant length in our case. Dijkstra's algorithm, conceived by Dutch computer scientist Edsger Dijkstra in 1956 and published in 1959[4] is a well known graph search algorithm that solves the single-source shortest path problem for a graph with nonnegative edge path costs, producing a shortest path tree. The Floyd–Warshall algorithm[5] is a graph analysis algorithm for finding shortest paths in a weighted graph (with positive or negative edge weights). A single execution of the algorithm will find the lengths (summed weights) of the shortest paths between all pairs of nodes though it does not return details of the paths themselves. The algorithm uses an adjacency matrix to determine the shortest path from every node to every other node. Initially this adjacency matrix contains the weight of the edge from node i to node j which respectively correspond to the row and column of the matrix, if there is no edge from i to j the weight is set to infinity. During these iterations the algorithm will check if there exists a shorter path between nodes. If this is the case it replaces the old value in the matrix with the new. In this way, after $(n)^3$ iterations we get all the shortest paths, where n is the number of nodes.

The Warshall algorithm was implemented in the AnalyzeSkeleton plugin. The maximum length of all shortest paths was used as output of the algorithm.

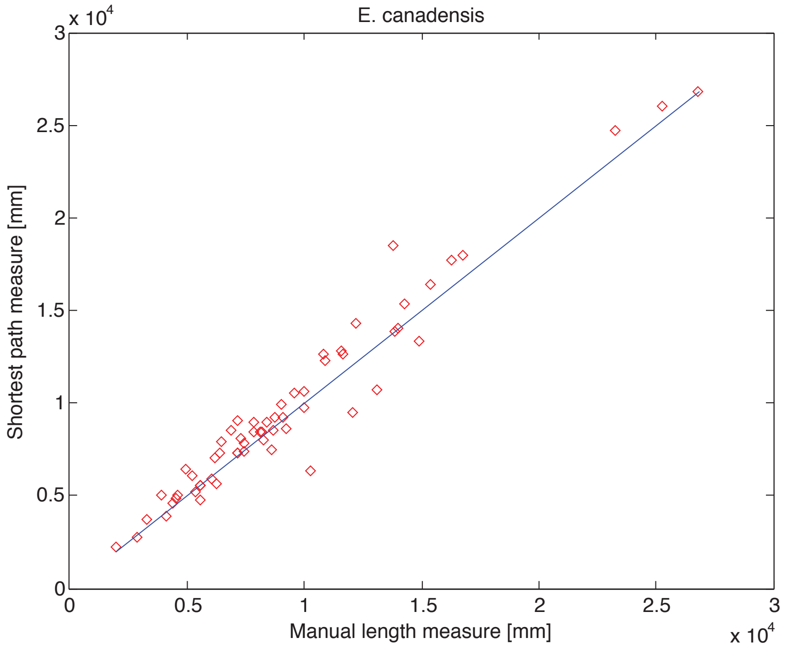
The Warshall algorithm does not return details about the paths, but in order to be able to verify the results it is nice when the shortest path is visualized. Therefore we reconstructed the found shortest path using the predecessor matrix which is computed at the same time as the adjacency matrix. In the predecessor matrix all the shortest paths going from node i to any other node are coded in row i. The column j then contains the predecessor node (hence the name) in the shortest path going from i to j. In this way we can reconstruct the longest path as drawn in the final image (figure 2(e)). In figure 3 we zoomed in on the upper part of figure 2(e). The longest shortest path is colored green.

4. RESULTS AND CONCLUSION

In order to test our approach the longest shortest path shoot lengths of 60 *E. canadensis* and 66 *M. spicatum* plants were compared with manual measurements. Figure 4(a) shows the manual measured versus automated measured shoot length of *M. spicatum*. The R^2 is 0.91 The results for *E. canadensis* are depicted in figure 4(b), with a R^2 of 0.94. From those high R^2 values we can conclude that the algorithm works very well.



(a)



(b)

Figure 4. Manual measured versus automated measured shoot length for *M. spicatum* (a) and *E. canadensis* (b).

M. spicatum shows one outlier which is possibly caused by a lost plant piece or segmentation error in case the plant touches the border of the image.

The total shoot length per plant is calculated by summing of the shoot lengths of all plant pieces in all images which were analyzed per plant. For each small piece a longest shortest path is determined. If there is a systematic error in the calculated length, this error will contribute more to the total error when the plant is cut in a large number of small pieces. Therefore it is advised to keep the plant pieces as long as possible.

The proposed procedure is a cost-effective approach to study concentration-response relationships of herbicides (and other relevant pesticides) on shoot length growth of submerged rooted macrophytes, as required in the test procedures underlying EU Regulation 1109/2009/EC.

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