A semi-automated image analysis method for benthic nematodes size assessment

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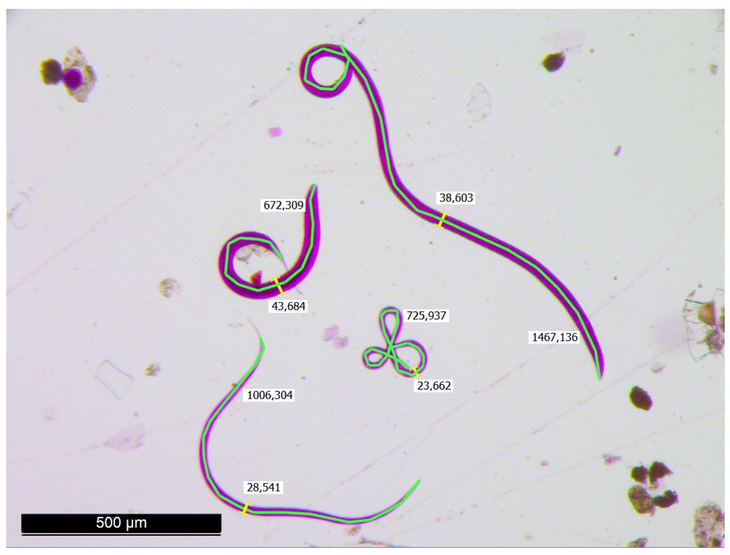
Introduction

Despite its ecological importance, meiofaunal size and biomass is rarely assessed in marine studies due to either time consuming and costly indirect procedures or inaccurate direct methods. Usually the volumetric approach based on Andrassy (1956) formula is used. It requires measurements of length and maximum width of each specimen, what makes it very time consuming and may be biased by interpersonal differences. Here, we present a semi-automated imaige analysis method of Nematoda measuremets allowing faster and more unbiased biomass estimates. The method was tested on samples collected in two habitats: seagrass vegetated sands (Baltci Sea) and subtidal muddy sediments (Spitsbergen fjords, Arctic).

Methods

Namatode isolation from sediments → Staining with Bengal Rose → Sieving on 500µm and 32µm → Photography of 100 random specimens per sample using Leica DFC450 digital camera

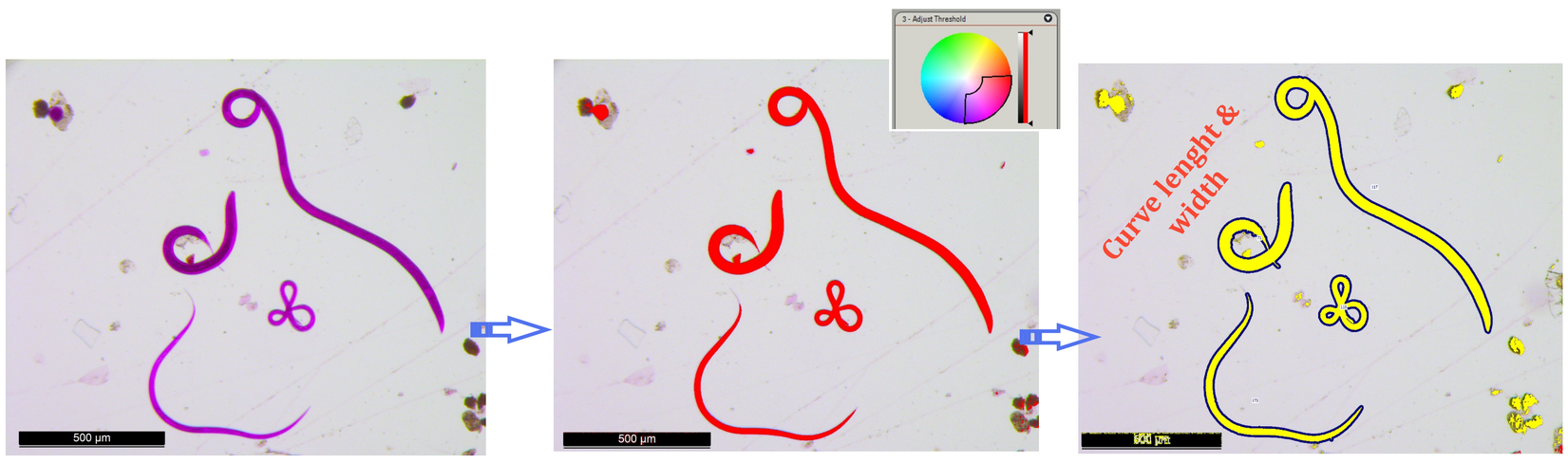
Manual measurements



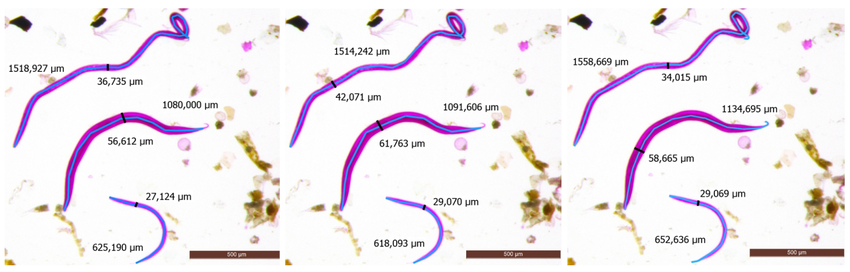
Semi-automated measurements

Using Leica LAS Image Analysis Module

Original image → **Color treshold selection** → **Image processing and measurements**



The same nematoda specimens measured by different persons



Biomass estimation

Biovolume → **Wet Mass** → **Dry Mass**

$V[\mu m^3] = \pi r^2 L / 10^6$

For semi-automated measurements

$WM\ [\mu g] = 1.13 \times V$

For manual measurements

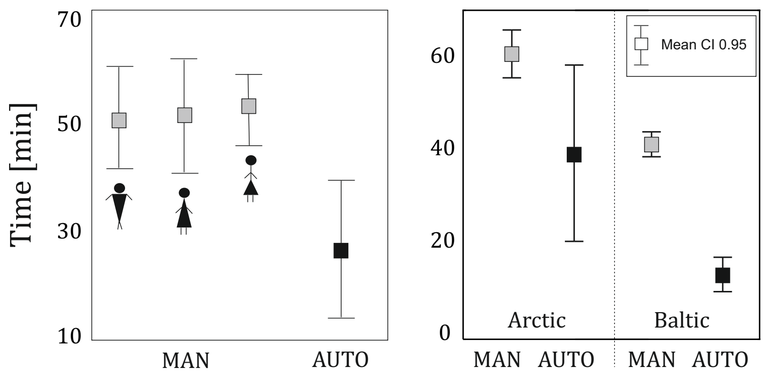
$WM\ [\mu g] = (L \times W^2) / Cf$

$DM\ [\mu g] = 0.25 \times WM$

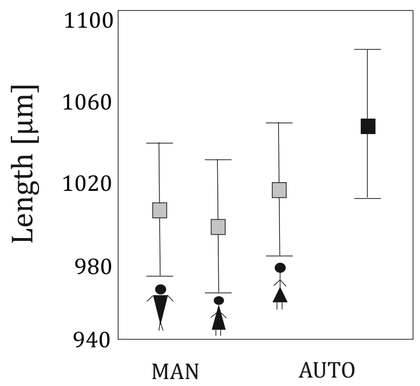
L - length, W- maximum width, Cf - conversion factor = 1.6×10^6

Results & Conclusions

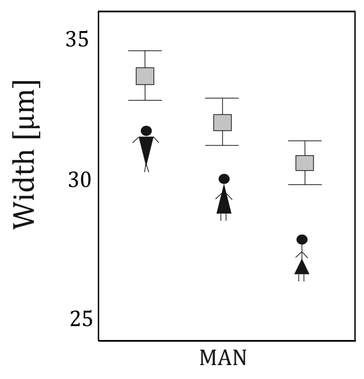
Time of manual vs. automated measurements



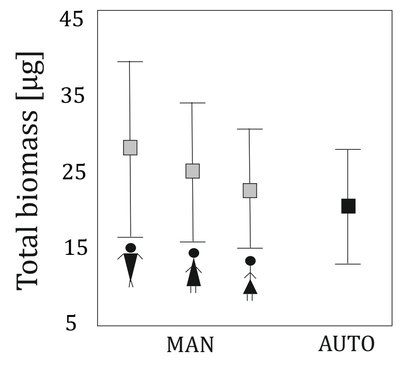
Nematode lenght



Interpersonal differences in manual maximum width measurements



Biomass estimations



- The semi-automated measurements are on average **2 times faster** then manual ones.
- There is **no significant difference**(Permanova $p>0.05$) **between length** measured manually and automatically
- Manual **width measurements differed** between analysts (Permanova $p<0.05$)
- The biomass estimated on basis of manual and semiautomated measurements is statistically similar (Permanova $p<0.05$)

Full method decription is available in: Mazurkiewicz, M., Górska, B., Jankowska, E., and Włodarska-Kowalczuk, M. 2016. Assessment of nematode biomass in marine sediments: A semi-automated image analysis method. Limnology and Oceanography: Methods, 14: 816–827, doi:10.1002/lom3.10128