Tracking and motion analysis of the cladoceran *Penilia avirostris*

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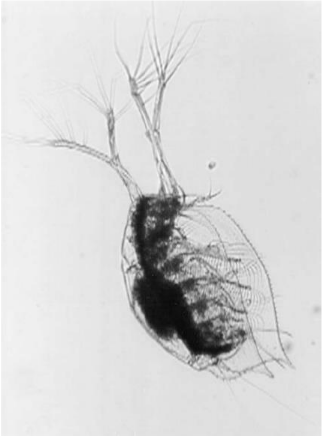
1. INTRODUCTION

- ***Penilia avirostris*** is one of the few marine cladoceran species, playing an important role in the zooplankton community of many tropical, sub-tropical and temperate waters ecosystems.

- **In the Gulf of Naples** (Southern Tyrrhenian Sea), *P. avirostris* has a typical seasonal distribution, with high abundances during summertime: in this season these crustaceans rapidly grow and develop, becoming dominant in coastal waters, and exerting a notable impact on the local phytoplankton community.

- The swimming behavior of *P. avirostris* is a typically **hop-and-sink** behavior.

- **Aim of the work**: to investigate the behavior of *P. avirostris*, in order to provide new insights into their **swimming abilities** in relation to **light and dark** changing conditions, trying to understand how physical factors as light can influence the behavior of this species.



Penilia avirostris

2. DATA AND METHODS

2.1. Sampling and video recording set up

1) **Zooplankton sampling:** on 30 September 2014 at the LTER-MC site (40° 48.5′ N; 14° 15′ E) in the coastal Gulf of Naples by vertical towing a Nansen net (200 μm mesh size).

2) **Selection of *P. avirostris*:** 20 individuals of *P. avirostris* were selected and placed into an observation chamber (1 L).

3) **Observational video setup:** designed and assembled at the Stazione Zoologica Anton Dohrn (Napoli, Italy). Two CCD video cameras (Sony XCD-X700), operating at 15 fps and mounting custom telecentric lenses, framed the central part of the observation chamber, with a field of view of 80×60 mm.

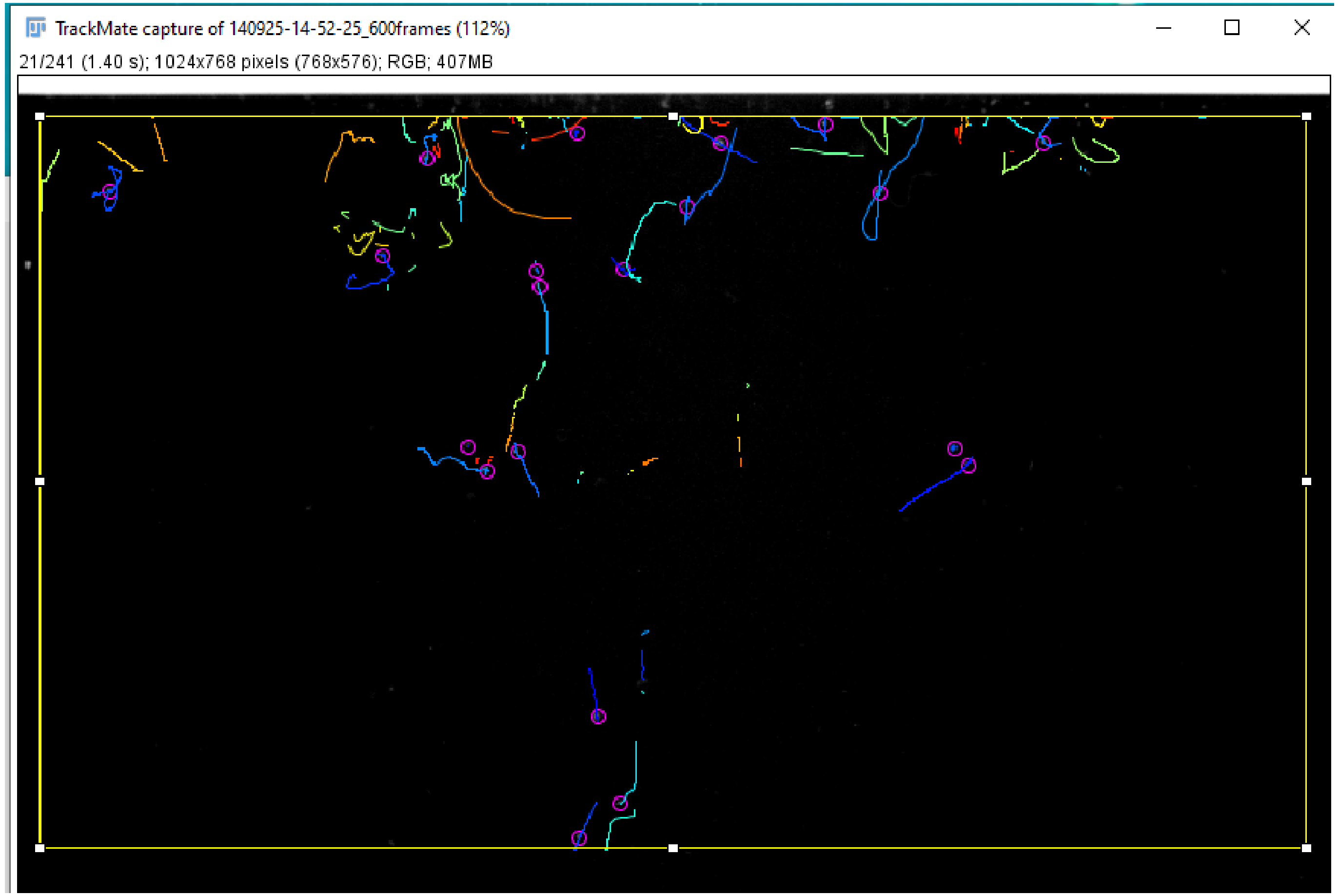
4) **Recording session:** The recording session consisted of three consecutive experimental conditions: 20 min in the dark (**D1**; IR LED panel only); 20 min in presence of white light from above the chamber (**L**); 20 min in the dark again (**D2**).

Location of Long Term Ecological Research-MareChiara station site (Gulf of Naples).

2.2. *ImageJ* software and *Trackmate* plugin

- ***ImageJ*** is a public domain image processing and analysis program, providing real world dimensional measurements.

- ***TrackMate* plugin**: it provides a way to automatically segment spots, and track them over time. It works like a fishing net with small holes: it finds as much spots as it can, even the ones not interesting, so there is a step to filter them out before tracking.



Examples of different trajectories of *P. avirostris*, obtained automatically from *ImageJ*.

2.3. Possibile sources of error during tracking activity

The software can **lose the point** to be tracked:

- the individual is too close to the border of the framing;
- the cladoceran image is not well clear and defined;
- the filmed animals move inside towards the inner parts of the observation chamber, so becoming out of the focus of the camera, or are too small to be located and tracked by the software;
- peaks of acceleration and relocation jumps are too fast to be followed by the software.

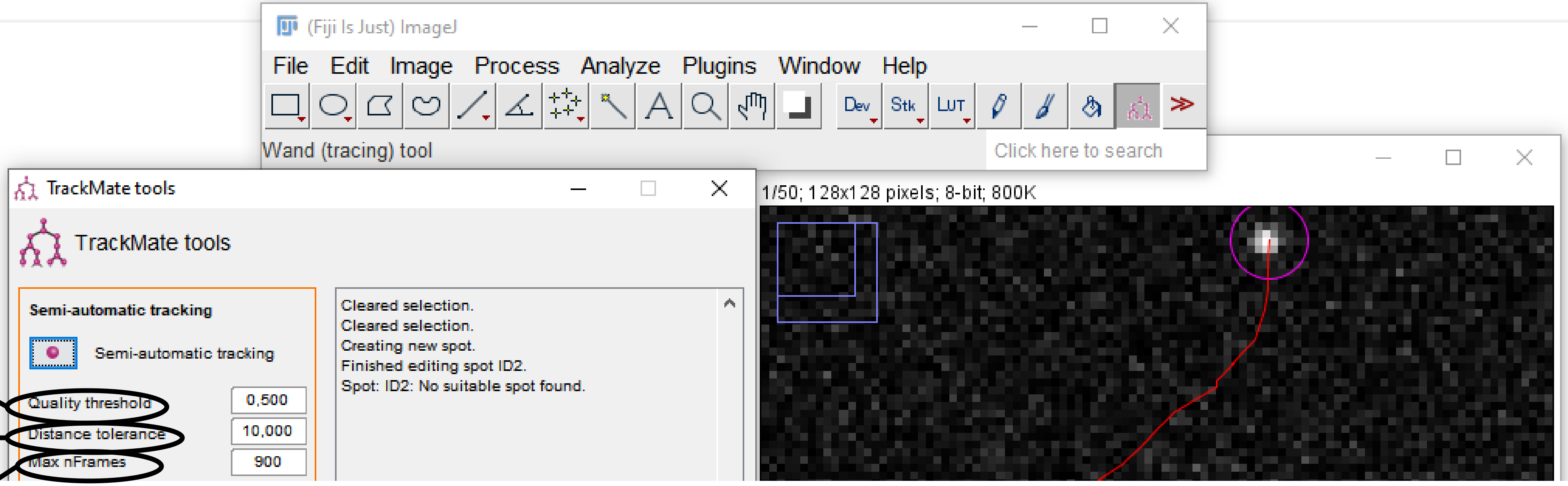
2.4. Semi-automatic method with *TrackMate* plugin

The **semi-automatic method**: allows the user to select manually a single spot and track it, and to intervene to correct errors in the detection of the trajectory. It takes a single spot selected by the user, and it uses its radius to search a neighbour spot in the next frame with a similar radius.

Quality threshold: 0.5

Distance tolerance: 10

Maximum number frame: 900



Example of a semi-automatic tracking with *TrackMate* plugin (on the left), and a portion of a trajectory (on the right).

2.5. Extraction of data

1) Manual selection of **10 individuals** for **every recording session.**

2) By using the semi-automatic approach, **tracking** for a time of approximately of 60 s. The software computed, for each spot tracked, the **position of the cladoceran in *x* and *y* component for every frame**, with a sample rate of 15 frames per seconds.

3) Extraction of **30 trajectories.**

4) **Conversion** of pixels into centimeters, with a conversion factor of 0.078, and frames in second, with a conversion factor of 0.07.

5) Each trajectory was characterized in terms of its **mean speed**, and the **autocorrelation** of the two speed components along *x* (*u* component) and *y* (*v* component) was calculated.

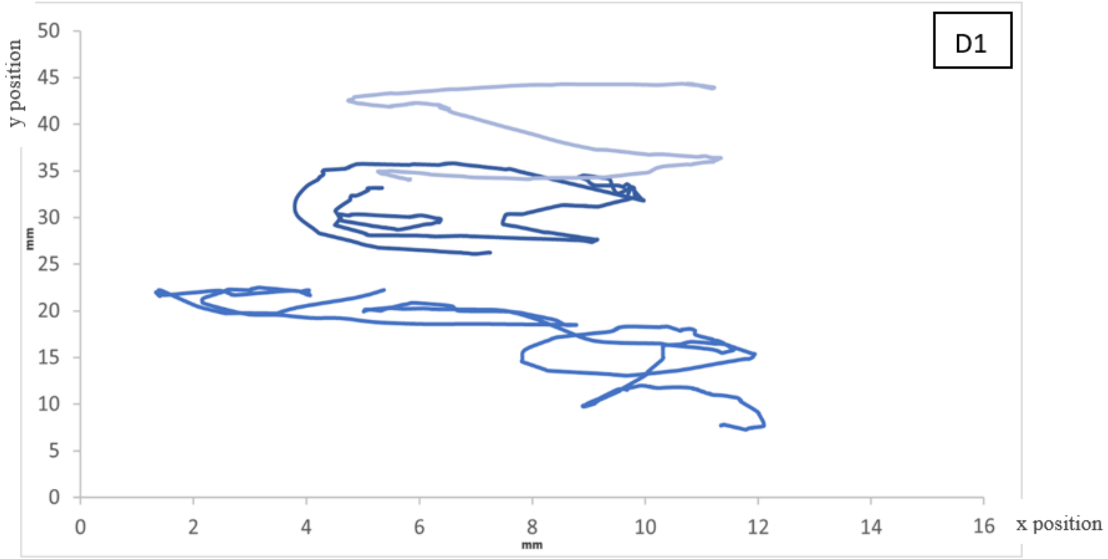
Recording sessions	D1	L	D2
Length of recorded videos	20 min	20 min	20 min
Number of trajectories extracted	10	10	10
Trajectory Duration	63.6 ± 2.4 s	54.5 ± 10.8 s	56.11 ± 11.0 s

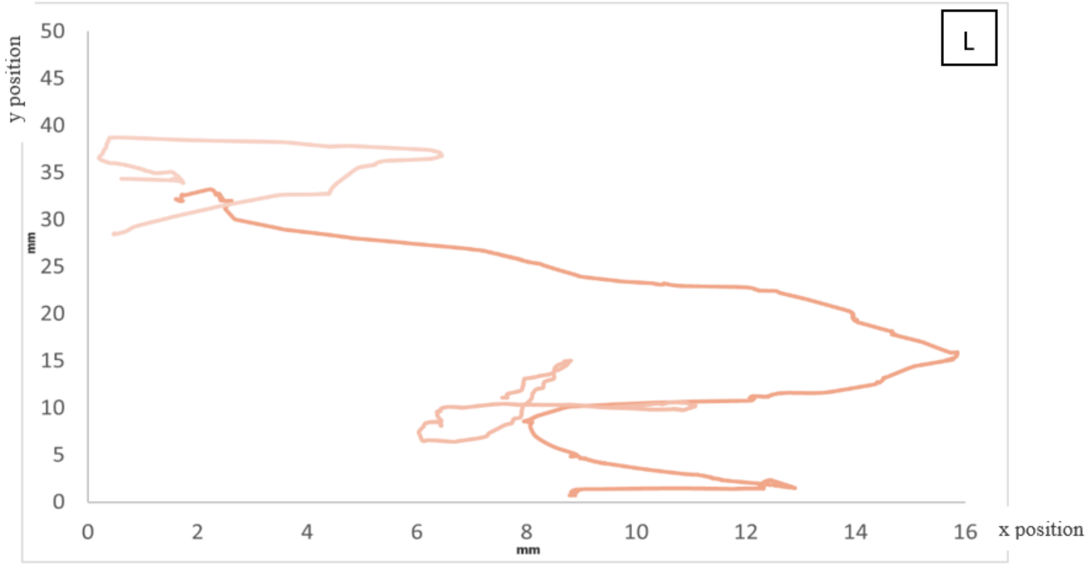
General information on *P.avirostris* swimming trajectories obtained in the presence and absence of light. The trajectory duration for each condition is indicated as mean ± sd.

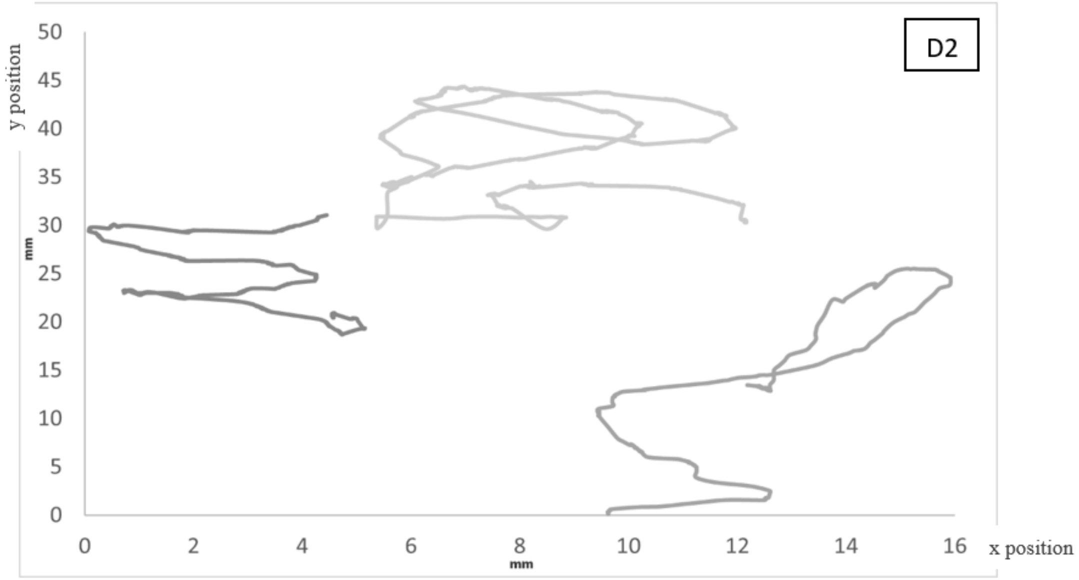
D1: dark condition
L: light
D2: dark after light condition

3. RESULTS

3.1. Trajectories



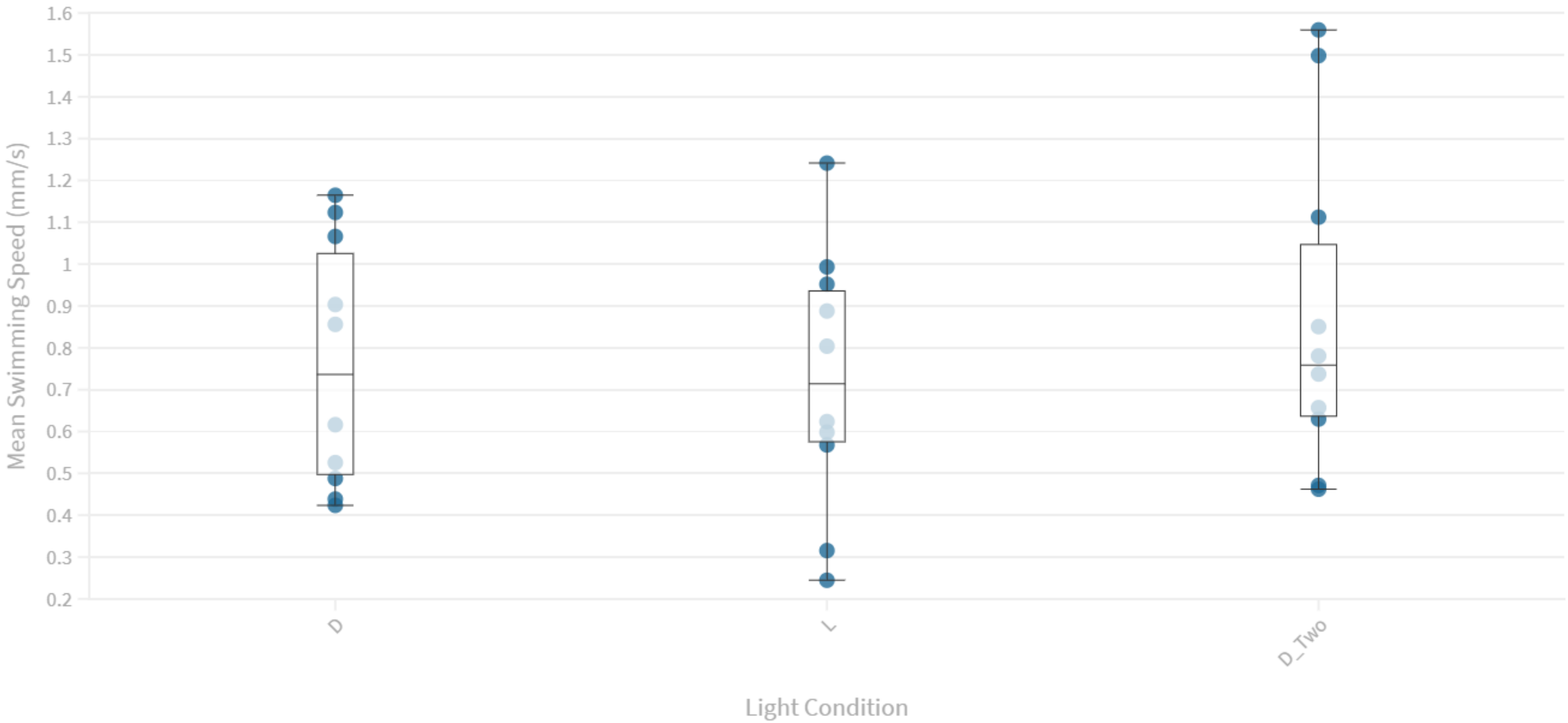




D1: dark condition
L: light
D2: dark after light condition

3. RESULTS

3.1. Speed measurements



Boxplots of the mean speeds (mm s^{-1}) in dark (D), light (L) and dark after light condition (D_Two).

- The mean speeds attained during the three conditions tested are similar, as their interquartile ranges overlap.
- The dispersion of values is minimal in D1, while it increases in the other two conditions, likely pointing to a higher degree of interindividual variability.

	Mean velocity (mm s^{-1})	Std (mm s^{-1})
D1	0.76	0.29
L	0.72	0.31
D2	0.87	0.39

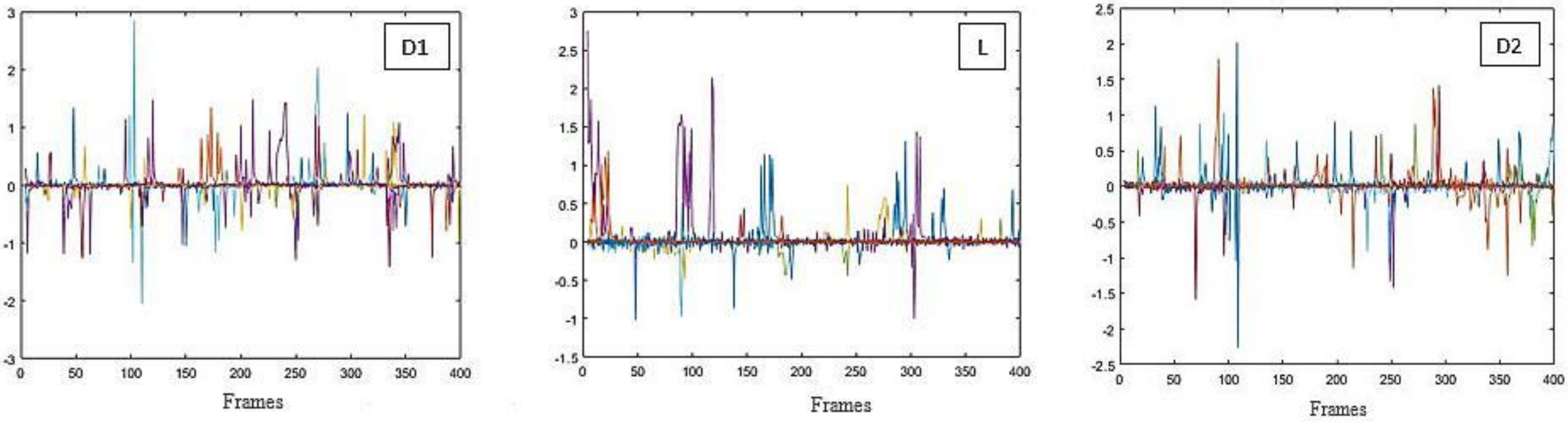
Mean of the individual mean speeds and standard deviation for the three sessions.

D1: dark condition
L: light
D2: dark after light condition

3. RESULTS

3.1. Speed measurements

- Alternation of irregular motion periods separated by a certain number of abrupt and rapid displacements towards different areas of the domain.

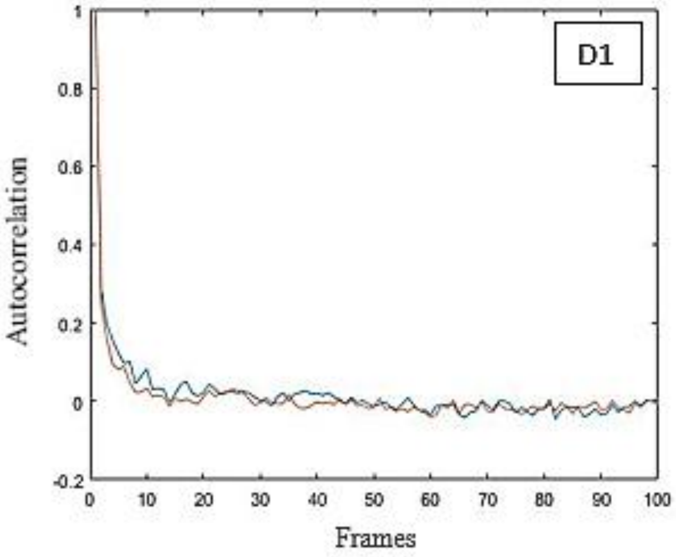


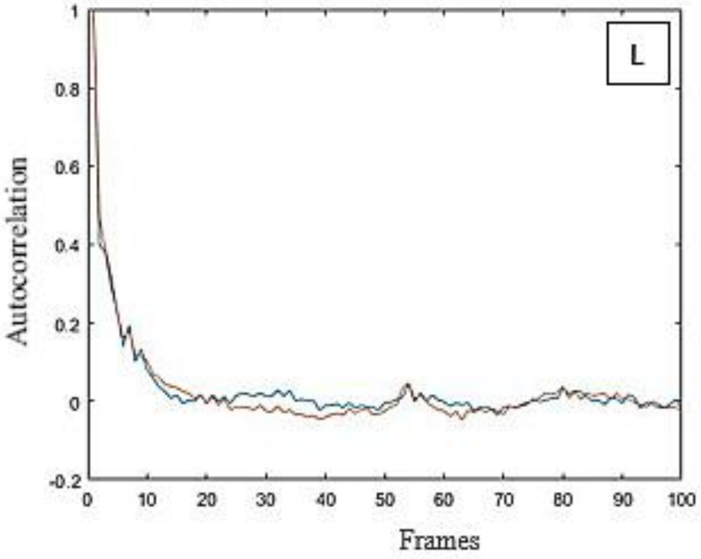
Time series of the *u* velocity component (cm s $^{-1}$) in the three different experimental conditions.

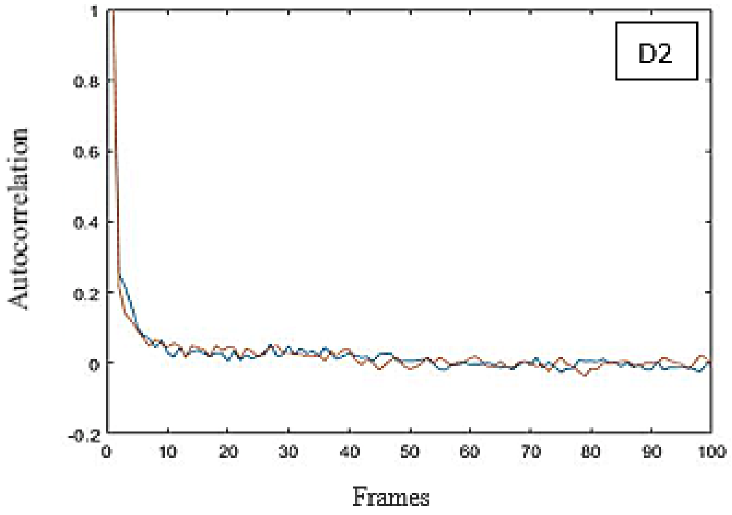
D1: dark condition
L: light
D2: dark after light condition

3. RESULTS

3.2. Autocorrelation







	Integral time scale
D1	
u component	0.105
v component	0.042
L	
u component	0.182
v component	0.14
D2	
u component	0.154
v component	0.161

Integral time scales (in seconds) along the *u* and *v* velocity components.

- The autocorrelation functions of the two velocity components *u* and *v* for the three different light conditions computed over the first 100 frames: fairly regular decreasing behavior, with different integral time scales.
- Integral time scale values: in dark conditions the drop in the memory is faster, while in presence of light the autocorrelation develops over relatively longer times.
- This could be due to a light-induced behavior, which reduces in part the erratic component of the behavior.

D1: dark condition
L: light
D2: dark after light condition

4. CONCLUSIONS AND FUTURE PERSPECTIVES

- The cladoceran attains **similar swimming speeds independent of the treatment**, but the memory of the motion is longer in presence of light, pointing to **an influence of light** in driving the movement.

- Starting from the first results collected, **future goals** can be envisaged in a deeper statistical analysis of the trajectories, and in ameliorations on the experimental setup, such as:

1) **optimization of the tracking method**, using other plugins, and trying to **reduce sources of errors** during the tracking process;

2) **increase in the numbers of trajectories** to extract;

3) implementation of the information about trajectories along the *z* axis, so obtaining **three-dimensional trajectories**.

Thank you