Tracking and motion analysis of the cladoceran *Penilia avirostris*

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Abstract

Plankton has a key ecological role in the marine environment as a core compartment of the marine food webs. Among zooplankton, the cladoceran *Penilia avirostris* plays a crucial role in the Gulf of Naples (Southern Tyrrhenian Sea), becoming dominant during summer and having an impact on the phytoplankton community. In this work, the abilities of *P. avirostris* to swim, live and adapt to a variable environment were investigated. In particular, the ability of these organisms to modify their swimming behavior upon different light conditions was tested in the laboratory. Twenty individuals were selected and placed into an observation chamber and filmed with two CCD (Charge-Coupled Device) video-cameras in three consecutive experimental conditions: in the dark (with the presence of a IR LED only), in presence of light, and in the dark again. Starting from these videos, the pivotal operation of this work has been the trajectories extraction using a tracking software, *ImageJ*. Thirty trajectories, ten for each experimental condition, were extracted with a semi-automatic method. The organism trajectories and their speeds show an irregular motion alternated with some abrupt and rapid displacements. The mean speeds of *P. avirostris* during the three conditions tested are quite similar, while the analysis of the autocorrelations of the two velocity components shows some differences between dark conditions, with an autocorrelation developing over relatively shorter times, and light conditions, in which a longer memory of the motion suggests light-induced behavior of these animals, probably due to an active role of light in driving the movement.

Key words: Cladocerans – *Penilia avirostris* – video-camera – tracking – trajectories – velocity measurements.

Introduction and objectives

Penilia avirostris (Dana, 1849) is one of the few marine cladocerans, playing an important role in the zooplankton community of many tropical, sub-tropical and temperate waters ecosystems (Marazzo and Valentin, 2003). This species is an important component of the marine food webs, because it makes the organic energy available to consumers at higher trophic levels, and controls phytoplankton biomass, feeding on a wide range of prey, but also on larger cells such as dinoflagellates and diatoms. Moreover, *P. avirostris* plays an important role in the recycling of nutrients in the upper water column (Atienza et al., 2006).

In the Gulf of Naples (Southern Tyrrhenian Sea), *P. avirostris* (Fig 1) has a typical seasonal distribution, with high abundances during summertime: in this season these crustaceans rapidly grow and develop, becoming dominant in coastal waters (Mazzocchi et al., 2011), and exerting a notable impact on the local phytoplankton community (Ribera d'Alcalà et al., 2004). Cladoceran behavior is influenced by numerous factors, such as changes in the light field, food concentration, temperature, presence of chemical substances (Uttieri et al., 2014). The swimming response to a stimulus is a typically hop-and-sink behavior, thanks to the use of second antennae, alternating upward movements with periods of rest (Uttieri et al., 2014). Cladocerans are able to move towards or away light: this migratory behavior is called phototaxis. During the daylight they perform downward migration, in order to avoid predators and damage caused by UV radiation.

The aim of this work is to investigate the behavior of *P. avirostris*, in order to provide new insights particularly 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.

P. avirostris individuals were collected in the Gulf of Naples and filmed in laboratory, in dark and light conditions, over 60 min. The principal activity of this master training internship has been to extract and to process data from this video, thanks to the tracking software *ImageJ*, and to provide a preliminary analysis of the experimental data obtained.

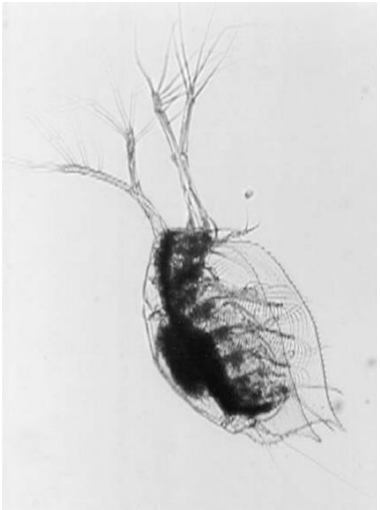

Fig 1 Image of the cladoceran *Penilia avirostris.*

2. Data and methods

2.1. Brief description of sampling and video recording set up

Zooplankton samples were collected on 30 September 2014 at the LTER-MC site (40° 48.5′ N; 14° 15′ E) (Fig 2) in the coastal Gulf of Naples (Southern Tyrrhenian Sea) by vertical towing a Nansen net (200 μm mesh size) mounting a non-filtering, glass cod end (5 L). Once in the laboratory (within a few hours from the collection), the samples were scrutinised and individuals of *Penilia avirostris* were manually picked using a large-mouth plastic pipette. The integrity of the individuals was checked by visual inspection under a Leica stereoscope.
20 individuals of *P. avirostris* were selected and placed into the observation chamber (1 L), filled with seawater collected at LTER-MC filtered on a 40 μm mesh to reproduce the natural food environment of the cladoceran while at the same time removing other zooplanktonic organisms. The observations were carried out at a water temperature of 22.5±0.5°C, the same temperature range experienced by *P. avirostris* during the sampling. The individuals were left to acclimatize 30 min before starting the observation session. The observational video setup was designed and assembled at the Stazione Zoologica Anton Dohrn (Napoli, Italy), and video observations were carried out using the 3D system described in Bianco et al. (2013). In short, two CCD video cameras (Sony XCD-X700), operating at 15 fps and mounting custom telecentric lenses, framed the central part of the observation chamber; each camera had a field of view of 80×60 mm, corresponding to the central part of the chambers (i.e., away from side walls, bottom and air-water interface). The chamber was illuminated through a 780 nm IR LED panel, using a wavelength which does not interfere with zooplankton spontaneous movement behavior. The recording session consisted of three consecutive experimental conditions: 20 min in the dark (D1; IR LED only); 20 min in presence of white light from above the chamber (L); 20 min in the dark again (D2). The recordings were realised between 14:20 and 15:20 (UTC+2), ensuring uniform circadian rhythms over the filming session.

Fig 2 Map showing the location of LTER-MC (Long Term Ecological Research-MareChiara) site, LAT = 40° 48.5′ N, LON =14° 15′ E, in the Gulf of Naples, where sampling has been done in 2014.

2.2. Extraction of data

As described before, the video recording has been divided in three sessions: 20 minutes of dark (D1, only IR LED was used), 20 minutes in presence of light (L), and 20 minutes of dark again (D2) (Table 1).
The core activity of this internship consisted in the extraction of data starting from these videos, using the software *ImageJ* (https://imagej.nih.gov/ij/index.html), a public domain image processing and analysis program, providing real world dimensional measurements. In order to extract trajectories, the plugin *TrackMate* (Tinevez et al., 2017) has been used, providing a way to automatically segment spots, and track them over time. It follows the classical single-particle tracking scheme, where the detection step and the particle-linking step are separated, therefore each step is handled in the user interface by a specific panel. Also, *TrackMate* 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.

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

Table 1 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.

As a preliminary step, videos had to be duplicated in '.tiff' format, compatible with the software, and converted in B&W collection of frames, in order to enhance the contrast between the background and the individuals, since the software works only with B&W images.
The initial procedure in *ImageJ* software is the selection of the detection algorithm to use. In this study, the LoG detector (Laplacian of Gaussian particle detection) algorithm for Gaussian-like particles in the presence of noise has been used. This detector is ideal for intermediate spot sizes, between 5 and 20 pixels in diameter, as in the case of *P. avirostris* in the recorded movies. The second step is the detection process, run automatically by the software to identify those particles to be tracked, and the spot filtering step, manually performed by the user to select the relevant spots only. After this manual filtering operation, the particle-linking algorithm has to be chosen. The Linear Assignment Problem (LAP) algorithm was used in the present study: it is well suited for particles undergoing Brownian motion, and it acts in two steps. First, it creates track segments from frame-to-frame particle linking; then it links track segments to achieve gap closing during the tracking process (Fig 3).
Possible sources of error that may be encountered during the tracking process are multiple. The software indeed can lose the point to be tracked, because of many reasons:

- 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.

Because of these errors and interruptions, the semi-automatic method provided by *TrackMate* plugin has been used. This approach allowed to select manually a single spot and to track it, and to intervene

to correct errors in the detection of the trajectory, should the software have encountered one of the possible tracking errors listed above.
The semi-automatic tracking tool works as follow: 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. The setting panel of the semi-automatic tracking (Fig 4) is composed by:

- the quality threshold: a quality threshold of 0.5 means that the spot found must have a quality of at least 50% the quality of the first spot to be accepted;
- distance tolerance: for instance, a distance tolerance of 10 means that the next spot must not be further away than ten times the radius of the previous spot to be accepted;
- max number frames: it sets a limit to the process. A maximum number of 900 frames has been set, in order to extract a trajectory of a minute duration. When the process stopped, the trajectory was printed in the log window.

Therefore, if the found spot is close enough and has a quality high enough, it is linked to the first spot. The process is then repeated, until no suitable spot can be found or until there is no time-point to inspect anymore.
By using the semi-automatic approach, 10 individuals were manually selected for every recording session, and tracked by the software for a time of approximately of 60 s. When the software lost the point, the tracking was done manually, giving the possibility to link, frame by frame, points of the trajectory lost by the software. The software computed, for each spot tracked, the position of the cladoceran in x and y component for every frame (corresponding to the horizontal and vertical position in the observation chamber), with a sample rate of 15 frames per seconds. A total of 30 trajectories have been extracted, and obtained data were converted in ASCII files (.txt) and pre-processed. Extracted data were cleaned through identification of outliers and spikes due to errors of the software, and smoothing them by using mean values.
Since position data were expressed in pixel and time in frames, it was necessary to convert pixels into centimeters, with a conversion factor of 0.078, and frames in second, with a conversion factor of 0.07. 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. Swimming speed provides an indication of how rapidly the organism moves in a given condition, whereas the autocorrelation indicates how long the movement at a given time t influences later motion, pointing at a memory in the behavior.

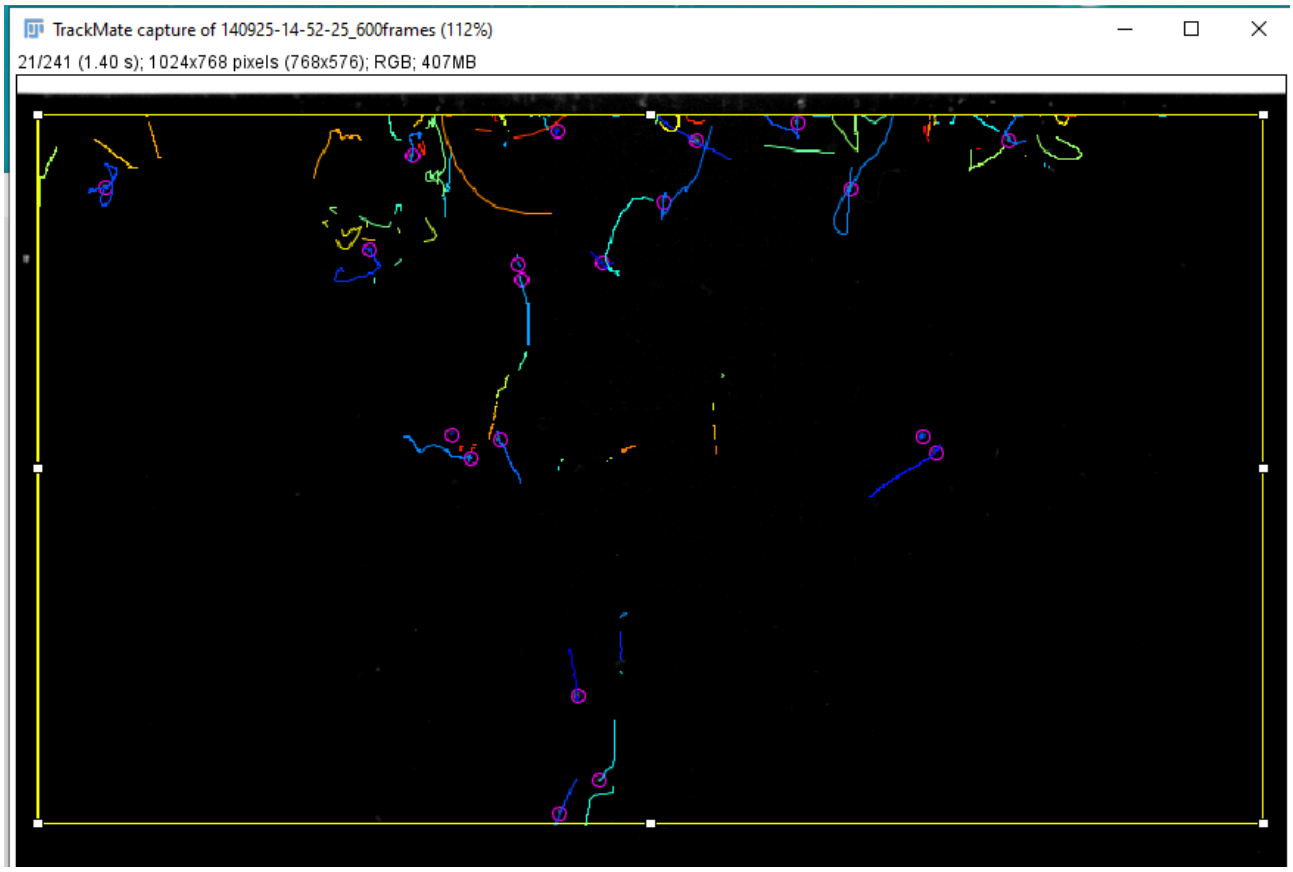

Fig 3 Examples of different trajectories of *P. avirostris*, obtained automatically from *ImageJ* software [source: personal picture].

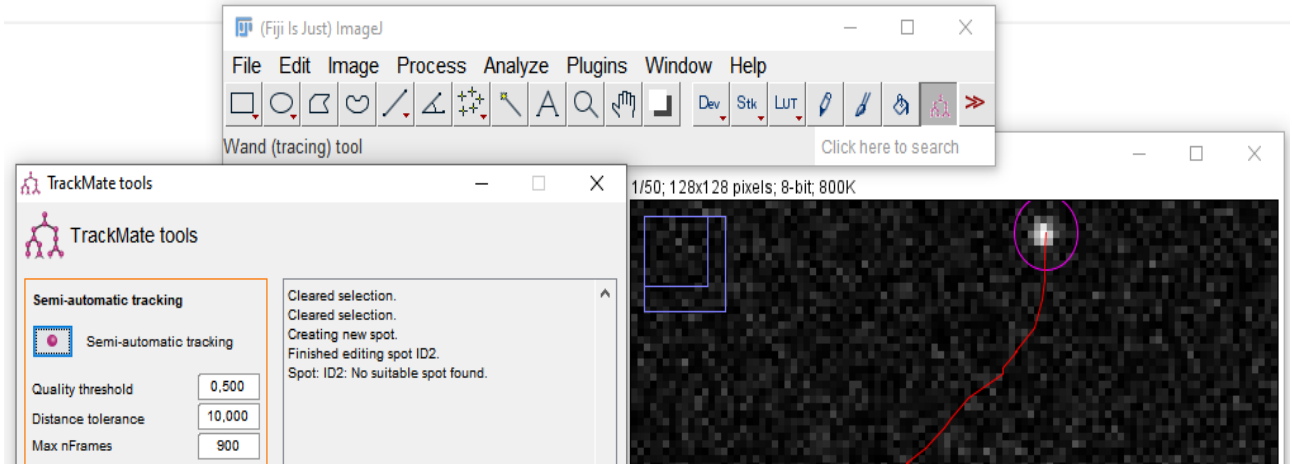

Fig 4 Example of a semi-automatic tracking with *TrackMate* plugin (on the left), and a portion of a trajectory (on the right) [source: personal picture].

3. Results and discussion

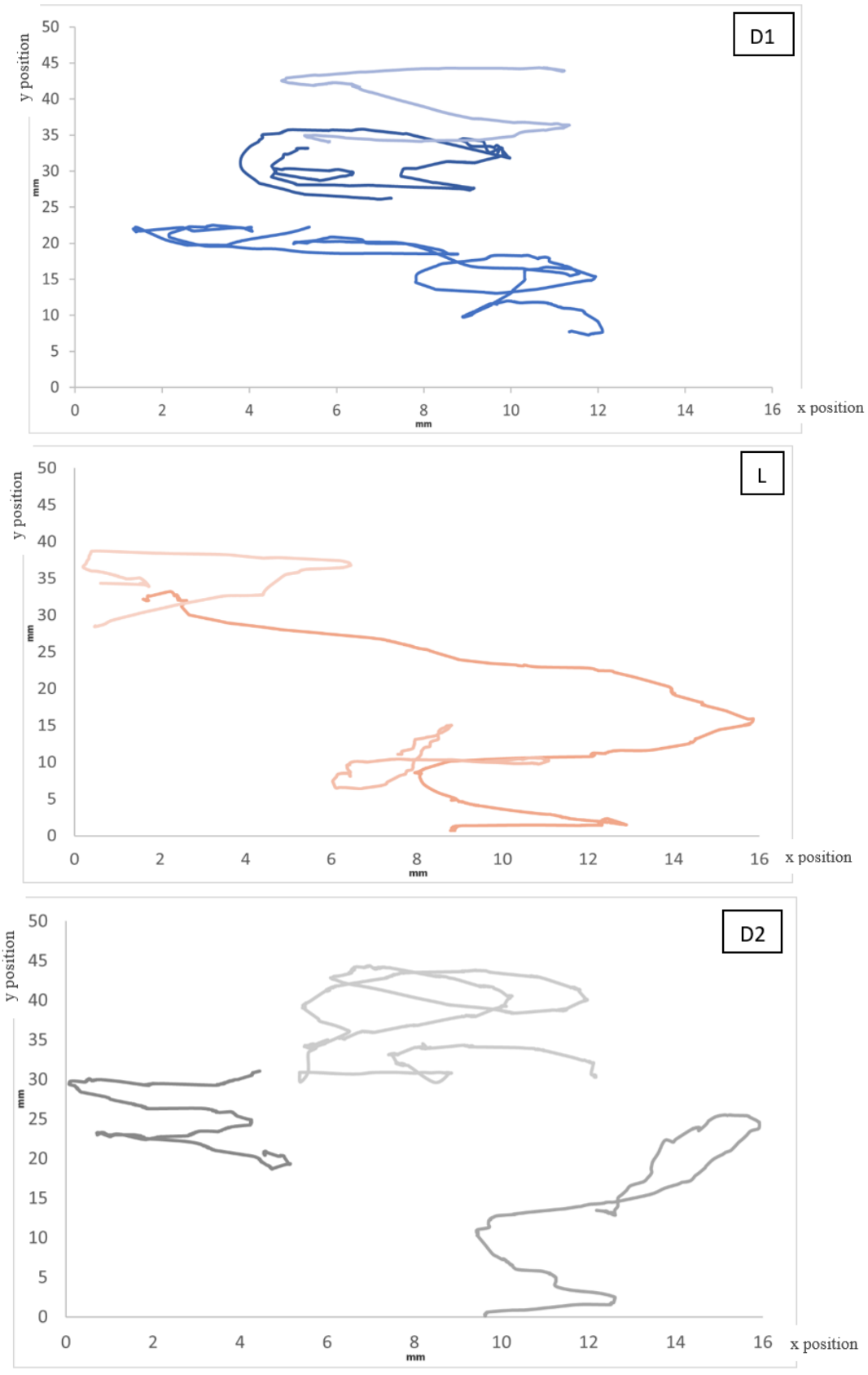

Fig 5 Examples of three different trajectories extracted with *Trackmate,* in the three sessions of dark (D1), light (L), and dark again (D2).

The swimming trajectories described by *Penilia avirostris* (Fig 5) were typically twisted and irregular, outlining a high variability of the movement of the cladocerans in the three different conditions. Table 2 provides a list of the mean speed of each trajectory in the three experimental conditions.

Track	Mean velocity (mm s^{-1}) D1	Mean velocity (mm s^{-1}) L	Mean velocity (mm s^{-1}) D2
1	0.90	0.95	0.63
2	1.16	0.60	1.50
3	0.44	0.99	0.85
4	1.07	1.24	0.78
5	0.86	0.80	0.47
6	0.62	0.31	1.56
7	0.52	0.62	0.65
8	0.42	0.57	1.11
9	1.12	0.89	0.74
10	0.49	0.24	0.46

Table 2 Mean speed (mm s^{-1}) of the ten trajectories extracted for the three recording sessions.

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

Table 3 Mean of the individual mean speeds and standard deviation for the three sessions.

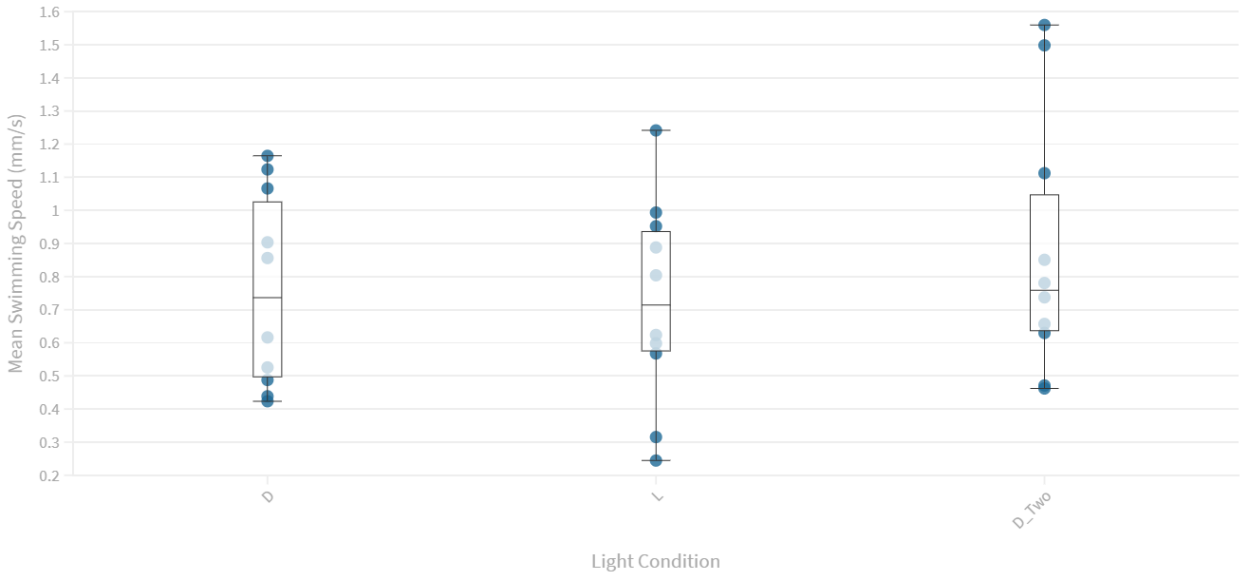

Fig 6 Boxplots of the mean speeds (mm s^{-1}) in dark (D), light (L) and dark after light condition (D_Two). Boxes represent the interquartile ranges, and whiskers extending from the two ends of the box indicate the dispersion of values below the first quartile and above the third quartile not classified as outliers.

Table 3 shows, for the three recording sessions, the three mean speeds and their standard deviations. For each session, a box and whisker plot is drawn (Fig 6): the bottom and the top of the box are respectively the 25th and 75th quartiles of the range, whereas the line inside it is the median. The length of the box is the interquartile range. Some features emerge from the analysis of the boxplots. Overall, the mean speeds attained during the three conditions tested are similar, as their interquartile ranges overlap. Notably, 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.
The organism trajectories and, consequently, speeds show an alternation of irregular motion periods separated by a certain number of abrupt and rapid displacements towards different areas of the domain. This is clearly illustrated in Fig 7, showing the time series of the *u* velocity component.
In order to gain some insight into the degree of self-correlation of the motion, i.e. on memory of the displacement process, the normalized autocorrelation functions of the two velocity components *u* and *v* for the three different light conditions were computed over the first 100 frames and are displayed in Fig 8. They all show a fairly regular decreasing behavior, with different integral time scales. This quantity, defined as the integral of the autocorrelation function, represents the typical memory time of the motion, and is obviously larger for processes characterized by a lower degree of randomness.

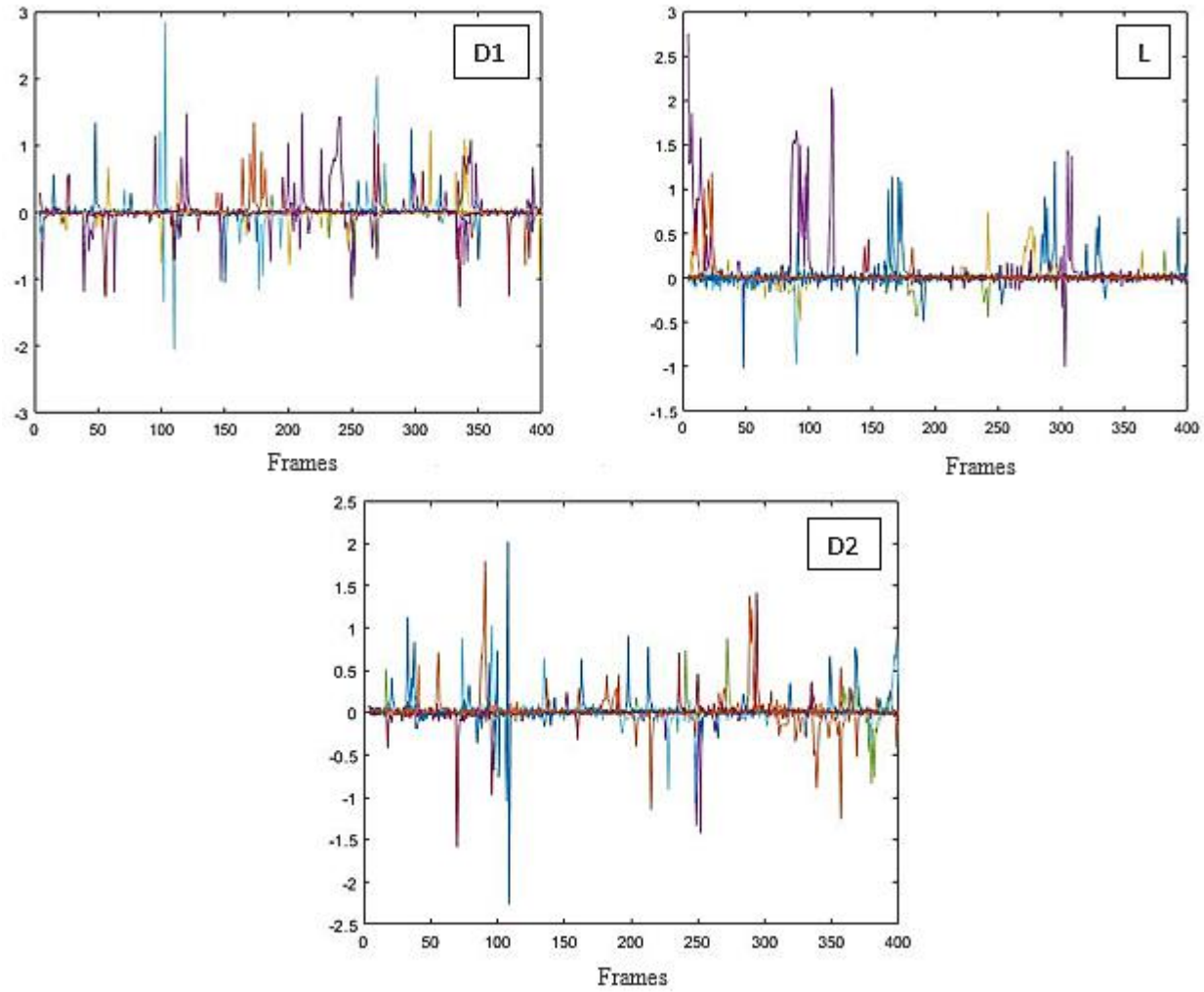

Fig 7 Time series of the *u* velocity component (cm s^{-1}) in the three different experimental conditions.

From visual inspection of Fig 8, as well as from the integral time scale values shown in Table 4, it can be noted that 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.

	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

Table 4 Integral time scales (in seconds) along the *u* and *v* velocity components.

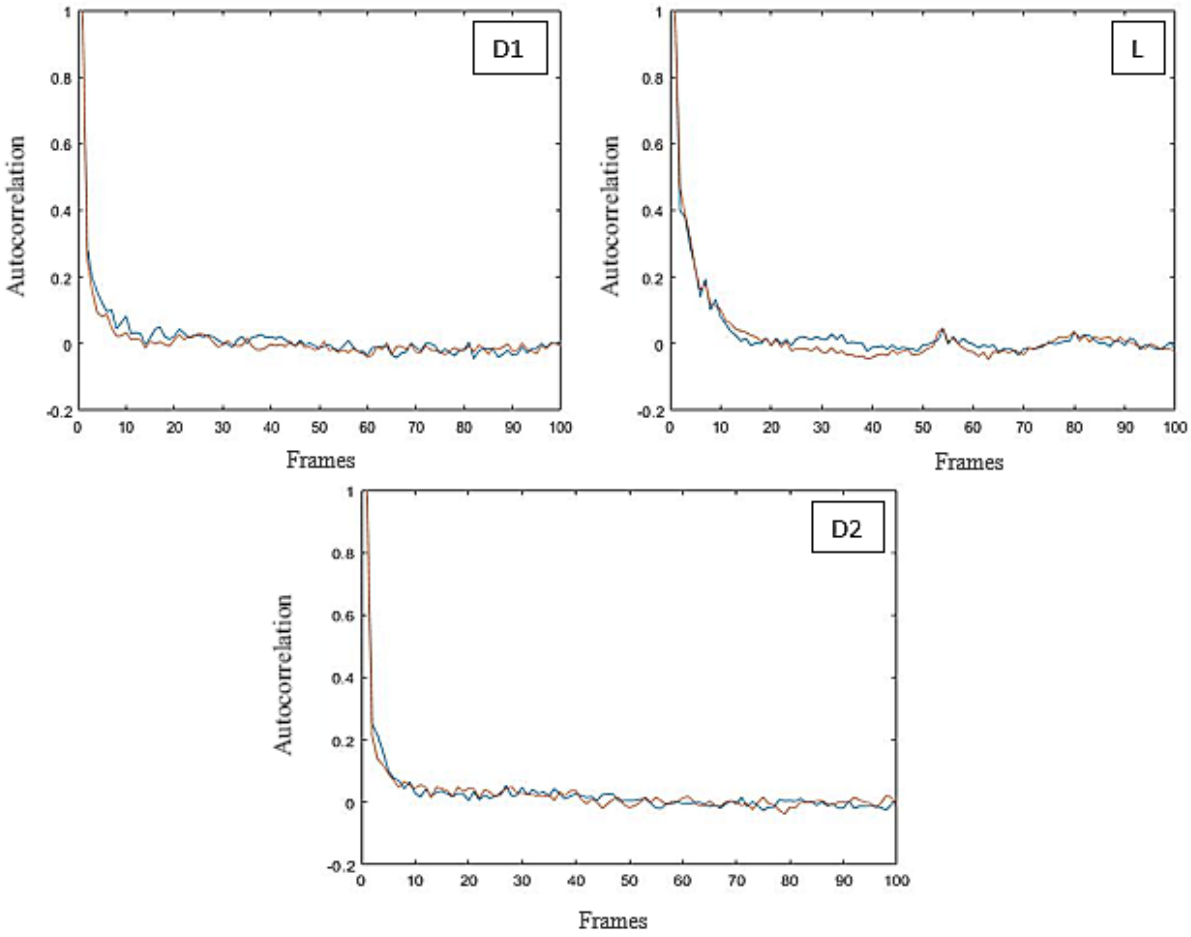

Fig 8 Autocorrelation of the two velocity components for the D1, L and D2 recording sessions.

4. Conclusions and futures perspectives

This internship aimed to investigate the swimming behavior of *Penilia avirostris* in relation to light and dark changing conditions, extracting position data from video filming. The thirty trajectories of tracked individuals analyzed in the present work show some key features in *P. avirostris* movement. 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 active role 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:

- optimization of the tracking method, using other plugins, and trying to reduce sources of errors during the tracking process;
- increase in the numbers of trajectories to extract;
- implementation of the information about trajectories along the *z* axis, so obtaining three-dimensional trajectories.

This future perspective will allow the deepening of the study on *P. avirostris* behavior, driving many ecological processes of the Gulf of Naples ecosystem.

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