

The tropical barnacle, *Balanus amphitrite*, is an economically important fouling species (it settles on natural and artificial submerged surfaces) with a short generation time and larvae that are relatively easy to culture in the laboratory. These attributes have contributed to its acceptance as a model species for larval settlement studies. The settlement stage larva - the cyprid (figure 1) - measures approximately 0.5 mm in length and is capable of swimming at high speed. On encountering a surface, the cyprid typically displays searching behavior, in which the cyprid 'walks' over the surface using the attachment discs of its paired antennules. This behavior has proved difficult to measure in detail by conventional means. Knowledge of such behavior is nevertheless important to the development of methods to interfere with this process, i.e. antifouling. The present investigation aimed to determine whether the settlement behavior of such small organisms was amenable to analysis by EthoVision and if so, to see how to optimize its use.



Figure 1. Interference contrast light micrograph of a cyprid larva of the tropical barnacle, *Balanus amphitrite*. The paired antennules used to explore the substratum are arrowed.

Experimental set up

The equipment was housed in a darkroom and comprised a digital color CCD camera, fitted with a Cosmimar-Pentax lens and connected to a PC and monitor (figure 2). The camera lens was placed 12.5 cm above the experimental arenas (35x10 mm Petri dishes). Several tests were conducted to optimize the video tracking protocol. Three arenas were used simultaneously with a single cyprid per arena. Petri dishes were placed on a backlight box and the subtraction method was applied to detect an object darker than the background (cyprids appear as small black points in the dish). Tracks were recorded at 25 samples/second. Under these settings [1], cyprids were readily detected by the software.

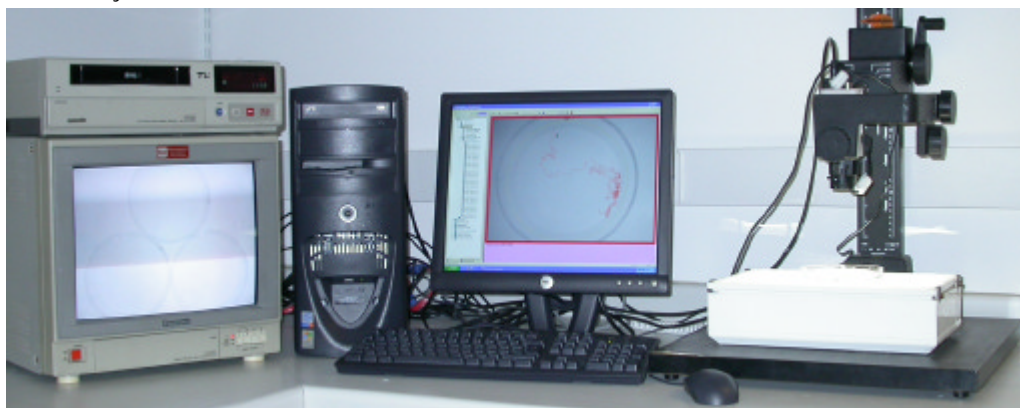


Figure 2. Video tracking equipment.

Data analysis

Several analysis profiles were used to calculate EthoVision statistics for cyprid behavior [1]: Distance (Parameters: Distance (total), Meander (mean, absolute); Filter: Minimum estimated distance moved), Turn angle + Velocity (Parameters: Turn angle (mean, absolute), Turn angle relative (mean, relative), Velocity (mean), Angular velocity (mean, absolute); No Filter), Movement (Parameters: Moving, Not moving (duration); Filter: Minimum distance

moved [0.001cm]). As small movements of the cyprid body, while stationary, could not be distinguished from movement over the substrate, the raw data were downsampled so that only every 12th point was sampled (downsampling step: 12). This was an acceptable time window to distinguish phases of settlement behavior visually. Raw data were also manipulated in Excel to analyze sub-behaviors.

Discussion

There are three phases to cyprid exploratory behavior: wide search, close search and inspection [2]. These sub-behaviors may be punctuated by periods of swimming or no activity. All these sub-behaviors could be extracted from EthoVision data. Sub-behavioral changes and their interrelation were highlighted by this analysis opening up the prospect for modelling and predicting behavior.

EthoVision behavioral parameter statistics were used to analyze and quantify the global behavior of cyprids subjected to different treatments. We accumulated valuable information on how larval behavior changes according to cyprid age or upon exposure to a cue to gregarious settlement. EthoVision also allowed us to assay compounds and natural biofilms (bacteria and macroalgal spores) for antifouling activity (figure 3). The tracking assay was complementary to a standard Petri dish 24-h settlement assay that is widely used in antifouling research. The former, being only of 5-min duration is well suited to assays of unstable compounds.

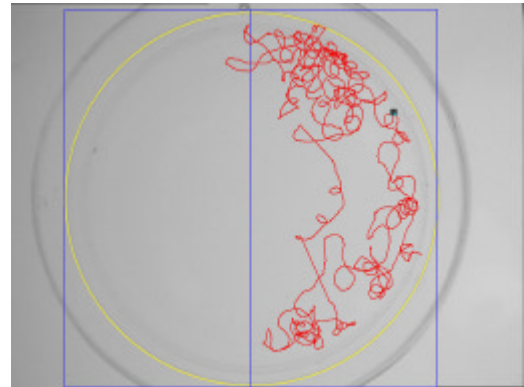


Figure 3. . EthoVision 5-min track of a single cyprid in a dish with an algal biofilm one half (left) and a clean surface on the other half.

Conclusion

The application of automated video tracking is a new approach to study marine larval settlement and is likely to find wide application in both fundamental and applied studies of this important life-cycle process.

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