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Real-time high resolution tracking of coral and oyster larvae

R.C. Geertsma, P. Kamermans, A.J. Murk, T. Wijgerde *

Wageningen University and Research, Marine Animal Ecology, P.O. Box 338, 6700, AH, Wageningen, the Netherlands

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ABSTRACT

Coral and oyster reefs have declined greatly due to anthropogenic stressors. Low recruitment rates from larvae hamper recovery of these important ecosystems. Although much is known about factors affecting larval settlement, a detailed understanding of their swimming and substrate selection behaviour is lacking. Here, we present an approach to study coral and oyster larval behaviour in unprecedented detail, using a high resolution camera, choice chambers and behavourial analysis software. From second-by-second spatial data, we extracted variables such as swimming pattern, swimming speed and distance travelled using larvae between 0.2 and 3 mm in length. We applied this to larvae of the Caribbean brooding coral *Favia fragum* and show they locate their major settlement cue, coralline algae, within 90 min when placed in a choice chamber. Oyster (*Ostrea edulis*) larvae exhibited reduced swimming speed with age, suggesting pre-settlement behaviour. With the presented real-time high resolution tracking approach we can address new questions related to the behaviour of coral, oyster and other marine larvae, with applications in ecology, aquaculture and coastal engineering. Most notable is future development of "flypaper" substrates with cues to promote larval settlement on reefs, to aid restoration efforts.

1. Introduction

Coral and oyster reefs have declined tremendously due to anthropogenic and natural stressors. For coral reefs, the broad-scale driver has been climate change (Eakin et al., 2019), although eutrophication, coastal development, sedimentation, storm damage, pollution, overfishing and destructive fishing locally contributed to reef deterioration (Aronson and Precht, 2016). For oyster reef losses, destructive fishing and overharvesting of oysters were the primary cause (Beck et al., 2011). Currently, both coral and oyster reefs show low recruitment rates from larvae, which hampers recovery and persistence of these important ecosystems (Beck et al., 2011; Hughes et al., 2019). For coral reefs, low recruitment rates have been explained by mortality of adult corals (Hughes et al., 2019) and competition with turf algae (Arnold et al., 2010). Most oyster reefs are so decimated that both larval supply and availability of hard substrates for settlement are limited (Beck et al., 2011).

Given their high economic and ecological importance, restoration of reefs is urgent. However, large-scale manual outplanting of corals or oysters on degraded or artificial reefs is costly, time-consuming, often has limited success and results in reduced genetic diversity when cloning techniques are used (Bayraktarov et al., 2016; Bostrom-Einarsson et al., 2020; Abrina and Bennett, 2021; Hynes et al., 2022). A more efficient alternative could be aiding recruitment through stimulating settlement of pelagic larvae released by existing brood stock. Although most offspring dies before reproductive age (Type III survivorship) due to suboptimal environmental conditions, competition and predation, some individuals may survive and contribute to more resilient reefs (Graham et al., 2008). To effectively enhance recruitment, a detailed understanding of habitat selection and settlement cues for larvae is needed. Although promising work in this field has been done during recent years, major knowledge gaps remain. Reef-building larvae may initially be attracted by hydrodynamic cues such as turbulence and waves (Turner et al., 1994; Fuchs et al., 2015), natural reef sounds (Vermeij et al., 2010; Lillis et al., 2013), specific combinations of light intensity and spectrum, and low water pressure (Gleason and Hofmann, 2011). Subsequently, microhabitat selection is mediated by biofilms with their suite of chemical compounds, as well as substrate colour and structure (Petersen et al., 2005; Birrell et al., 2008; Nozawa, 2008; Erwin and Szmant, 2010; Mason et al., 2011; Mesias-Gansbiller et al., 2013; Tebben et al., 2015; Whalan et al., 2015; Gomez-Lemos et al., 2018; Jorissen et al., 2021). A functional lure for settlement in the field, however, has not yet been developed and it also is unknown how biochemical, visual and structural cues interactively affect settlement. In addition, the combined effects of anthropogenic stressors, including elevated water temperature, ocean acidification and pollution (e.g. nutrients and UV-blockers from

* Corresponding author.
E-mail address: tim.wijgerde@wur.nl (T. Wijgerde).

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sunscreens) on detection of cues by reef-building larvae are still unknown.

To study the effectiveness of settlement cues and substrata under current and future environmental conditions, multi-factorial experiments are required to unveil interactions across various cues, substrates and stressors. This requires an efficient, small scale and high-resolution approach to test behavioural effects on very small larvae (0.2–3 mm in length) of biological and chemical settlement cues under varying environmental conditions. With a real-time high resolution tracking system for coral, oyster and other marine larvae, timely questions can be addressed and experimental "flypaper" substrates developed with cues to promote larval settlement in order to aid reef restoration efforts.

Here we present such a system, which consists of a high-resolution camera with choice chambers and third-party behavioural analysis software. This tool was inspired by a commercially available tracking system with lower camera resolution. In two separate pilots, we analyzed larval behaviour of a Caribbean brooding coral (*Favia fragum*) and the European flat oyster (*Ostrea edulis*). From second-by-second spatial data, we extracted swimming pattern, swimming speed and distance travelled by these larvae of 0.2–3 mm in length. We show that *F. fragum* larvae locate their major settlement cue, coralline algae, within 90 min when placed in a choice chamber.

2. Materials and methods

2.1. Animal collection

To establish a breeding population for coral larvae, 15 adult Caribbean Golfball coral (*Favia fragum*) colonies were collected by SCUBA between 3 and 5 m depth around the Sea Aquarium, Willemstad, Curaçao in July 2019. These corals were hand-picked by removal with a small chisel, labeled, and individually placed in ziplock bags underwater. After collection, corals were transported to CARMABI Institute (Willemstad, Curaçao) and placed in aquaria with running seawater for acclimation. For transport to Wageningen University, corals were individually placed in 1 L ziplock bags, with 30/70% seawater/air. A secondary ziplock bag was placed around the primary bag, to increase isolation and security. All bags were stacked in a 30 L isolated box and shipped in a heated cargo hold. Collection was permitted by the CARMABI institute, and transport was done under CITES export permit no. 19CW002 and import permit no. 19NL270335/11. After transport, corals were temperature-acclimated over a two-hour period to 28 °C in a 300 L aquarium at CARUS Aquatic Research Facility (ARF) of Wageningen University (see *husbandry* for details). Transport water was discarded.

Flat Oyster larvae (*Ostrea edulis)* were acquired via two hatcheries, Roem van Yerseke B.V., Yerseke, The Netherlands, and Stichting Zeeschelp, Kamperland, The Netherlands. Larvae were transported in 10 L plastic flasks containing ~30 ppt filtered seawater at a temperature of ~20 °C. During transportation by car to Wageningen in June 2021, the flasks were stored in a styrofoam box. Larval size varied between 220 and 280 μm (umbo and pediveliger stages), and their age varied between 10 and 16 days post release (dpr) by their parent oysters. To provide feed for the oyster larvae, live phytoplankton mixtures (*Isochrysis galbana* and *Chaetoceros calcitrans* from Roem van Yerseke and *Isochrysis* T-strain, *Pavlova lutheri, Nannochloropsis oceanica, Rhodomonas salina, Chaetoceros calcitrans, Thalassiosira pseudonana, Thalassiosira weisfloggi, Skeletonema costatum, Skeletonema marinoi* from Zeeschelp) were simultaneously transported in plastic flasks.

2.2. Husbandry and larval collection

Corals were maintained in a 300 L glass aquarium with the following daily-checked target parameters: temperature 28 °C, Salinity 36 ppt, alkalinity 2.5 mEq L^{-1}, calcium 420 mg L^{-1}, nitrate 0–1 mg L^{-1}, orthophosphate 0.02–0.1 mg L^{-1}. Calcium and alkalinity were maintained using regular additions of calcium chloride dihydrate and sodium bicarbonate, respectively. In addition, 30% of the aquarium water was exchanged three times a week using artificial seawater produced from deionised water (reverse osmosis) and Zoo Mix sea salt (Tropic Marin GmbH, Germany), which was aerated for at least two days prior to use. Corals were provided with <24 h old *Artemia* nauplii daily at a final density of 30–100 individuals L^{-1}. Two LED fixtures (CoralCare generation 1, Philips, The Netherlands) provided a parabolic light regime with 12 h photoperiod (9:30–21:30 h) and a peak irradiance of 350 μmol quanta m^{-2} s^{-1} (~400–700 nm). Irradiance was measured in situ with a Li-Cor using a LI-COR 192SA quantum underwater sensor (LI-COR, USA). After 1 month of acclimation, coral colonies were placed in a larval collection system several times per week around 16:30 h. No lunar light cycle was provided. Water flow was provided using a pump (Turbelle nanostream 6045, Tunze, Germany) with a flow capacity of 4500 L h^{-1}. Water filtration consisted of a 20 W UV filter (AquaHolland, The Netherlands) and a protein skimmer (MCE 600, Deltec, Germany).

Coral larvae were collected from adult colonies using a custom-made flow-through system (Geertsma et al., 2022), which consisted of 8 one-liter measuring beakers each holding an individual adult colony. Aquarium water was provided by a pump (Silence 300–3000 L h^{-1}, Tunze, Germany) delivering aquarium seawater into a 20 mm PVC pipe which branched out to each beaker via a 6 mm plastic hose. The seawater flowed over the beaker handles into collection sieves (mesh size of 120 μm) of which the bottom half was submerged. From this water layer, coral larvae which had been released by their parent colonies early in the morning (before the aquarium lights turned on) were collected between 10:30 and 11:00 a.m. The advantage of this setup is that larvae are not pumped through any pipes or tubing thus preventing damage. They also are provided with a continuous gentle supply of seawater of stable salinity and temperature, originating from and draining back into the main aquarium.

Within one hour after the aquarium lights had turned on, larvae were removed from the collection sieves using plastic Pasteur pipettes and individually placed in glass test tubes filled with 15 mL 0.5 μm filtered artificial seawater (FASW). Test tubes were placed in a water bath to maintain ambient temperature of 28 °C, at the same light regime as their parental colonies. Next, 54 tubes were prepared with FASW and one larva was placed per tube. Approximately 90% of the seawater was exchanged daily to prevent bacterial growth. Test tubes containing larvae were randomly assigned to 3 treatments to be tested in the choice chamber at 0, 1 and 2 days post release (DPR).

Oyster larvae were housed in two spherical glass aquaria of 5 L, filled with artificial seawater and fed daily with several mL of the live phytoplankton culture until a light green haze was visible, matching approximately 40,000 cells per ml. The aquaria were gently aerated using an air pump connected to a 5 mL serological pipette, resulting in individual air bubbles softly running along the side wall of the aquaria. The phytoplankton culture was maintained separately in 30 ppt aerated filtered natural seawater and 20 °C for several days while experiments lasted.

2.3. Larval tracking systems

For coral larvae, an off-the-shelf tracking system was used as a basis for our setup (DanioVision, Noldus, The Netherlands). This system was equipped with a camera (GeniCam, Basler, Germany) which recorded images at 1280 × 853 pixels, at 25 frames per second (fps). The camera and lens were mounted in a dark enclosure, allowing for recording of larval silhouettes without water reflection. The DanioVision choice chamber had dimensions of 127 × 82 × 10 mm (length x width x height) and contained 6 individual lanes of 110 × 10 × 9 mm and 8.5 mL water volume. The DanioVision chamber was equipped with a water bath to maintain constant temperature, which was set at 28 °C for the coral larvae.

The DanioVision was not suitable for the much smaller flat oyster

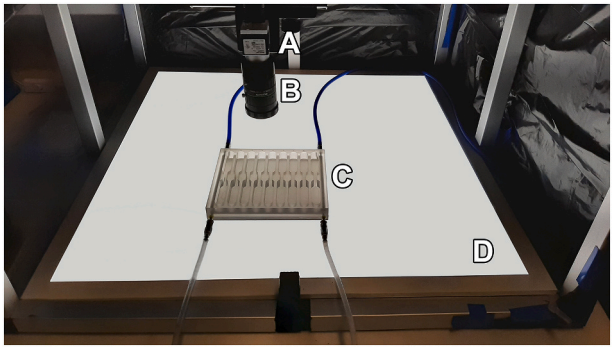

Fig. 1. Setup for the study of oyster larvae, with 20 Megapixel Basler camera (A), 12 mm Fujinon lens (B), acrylic choice chamber having 9 individual lanes plus internal water jacket (C) and LED panel providing uniform back light (D). The camera pointed downwards directly above the choice chamber, and was connected to a Windows computer via a USB 3.0 cable. A 3D model of the choice chamber is available as Supplementary Fig. 1.

larvae (220–280 μm compared to the 1500–3000 μm long coral larvae). Therefore, a second tracking system was custom-built. This system consisted of a high resolution camera (acA5472-17uc, Basler, Germany), a 12 mm Fujinon lens (CF12ZA-1S, Fujifilm, Japan) and an acrylic 9-lane choice chamber. This chamber was produced by the technical services of WUR, with dimensions 160 × 140 × 20 mm and 5 mL water volume per lane (Supplementary Fig. 1). A climate control water bath was integrated in the chamber and a LED panel (40 W, 60 × 60 cm, with 4000 lm of total light output at a colour temperature of 6000 K) provided uniform back light from below (Fig. 1). Camera and lens (Fig. 1A-B) were mounted right above the choice chamber and pointed downwards in a blinded enclosure, obscuring stray light from outside. The camera recorded images at 3200 × 2800 pixels at 10 fps and was connected to a Windows computer via a USB 3.0 cable. Photosynthetically Active Radiation (PAR) was measured at 100 μmol m^{-2} s^{-1} with an inverted LI-COR 192SA quantum underwater sensor (LI-COR, USA) directly on top of the filled choice chamber. In practice, only the middle 6 choice lanes were used as the Fujinon lens could not properly resolve the edges of the outer lanes. The choice chamber (Fig. 1C) was fitted with a water jacket connected to four 6 mm plastic tubes, through which fresh water was pumped with a return pump (300 compact, Eheim, Germany). The pump was submerged in a 20 L reservoir which was kept at 20 °C (oysters). A 3D model of the chamber is available as Supplementary Fig. 1.

2.4. Experiments

Coral larvae were tracked in the DanioVision. Since these larvae are attracted to chemical cues that crustose coralline algae (CCA) and their associated microbiota release (Ritson-Williams et al., 2009; Petersen et al., 2021), 0.5 × 0.5 cm CCA chips (*Hydrolithon* sp., hereafter named chips) were cut daily from coral rubble (live rock) originating from Curaçao with the *F. fragum* colonies. Chips were kept in the aquarium and allowed to recover for 24 h prior to testing, to prevent release of potential toxic metabolites as a result of fragmentation. Larvae were introduced in the middle of each lane, with one larva being released per lane. After a 15 min acclimation period, CCA chips were placed alternatingly on the left or right side of each lane, leaving the other side empty. Although this approach did not allow us to determine what type of cues (chemical, structural or visual) offered by the CCA chips resulted in larval attraction, it was suitable for generation of tracking data and heat maps, i.e. to determine whether larval attraction could be detected using our setup. Three 90-min runs with 1 larva in each of the 6 lanes per run were performed daily for three consecutive days, yielding 18 independent replicates per day (0, 1 and 2 dpr). In between runs, the lanes were rinsed twice with FASW. At the end of each experimental day, the chamber was thoroughly scrubbed with a clean tooth brush and rinsed with deionised water, followed by 70% ethanol rinsing and another deionised water rinse. The chamber's acrylic surface was very smooth and inert, thus likely retaining no chemicals or biofilms after a run.

For oysters, a similar approach was used, using the custom-built setup. Chips of oyster shells and limestone were used as potential cues, as they generated favourable settlement results with *O. edulis* larvae in prior trials (unpublished data) and other *Ostrea* species (McAfee and Connell, 2020). Larval responses to these substrata were compared against those to the empty control side, or directly compared against one another by placing them together on opposite sides of the lanes. Four runs, each with 6 individual larvae were performed, at 14, 15, 16 and 19 dpr, yielding 6 independent replicates per day. Days 17 and 18 were not recorded due to time constraints. Oyster larvae were fed prior to experiments to darken them, making tracking more accurate. When larvae were added in the narrow middle area, the software (see *data analysis*) failed to notice them. Thus, larvae were randomly added on one side as close as possible to the middle. In the same way as for corals, one larva was released per lane, using 6 lanes per run, and chip placement and data collection started after a 15 min acclimation period.

2.5. Data analysis

Tracking videos of larvae were analyzed using EthoVision XT 16.0 (Noldus, The Netherlands) and their position assigned to either the left, middle or right side of the choice chamber. This resulted in 90 min × 60 s × 25 fps = 135.000 data points generated per coral larva in the DanioVision, and 90 min × 60 s × 10 fps = 54.000 data points per oyster larva. Data were checked for outliers using track editor, and considered 'good' if >90% of the datapoints were recorded. When tracking quality fell below 90%, the video was opened in track editor and manual corrections for missing or incomplete datapoints were made. In track editor, datapoints are projected over the video recording, allowing for manual correction and interpolation of missing data points. Such points may occur when a larva has crept underneath or very close to a substrate, rendering it invisible to the camera. After correcting data points, tracks were reanalyzed. From the raw data, individual swimming speed and swimming distance over the 90-min period were extracted. Stationary larvae were excluded from swimming speed calculations.

The total number of data points spent in one of the three position options was divided by total datapoints spent in the chamber. Since few larvae spent time in the middle and this is merely a transitional zone, for statistical analysis purposes, time spent on the left and right side of the choice chamber were used. For coral larvae, data was normally distributed (Shapiro-Wilk, $p > 0.05$) since a randomly swimming larva without cues spends its time equally (50–50%) divided over both left and right sides. A one-sample *t*-test was performed with 0.5 as the reference value at an alpha of 0.050. A one-sided p was used to test the hypothesis that CCA chips attract coral larvae, with more time spent on the side containing CCA chips. For oysters, insufficient movement between lane sides was found, with larvae often remaining on either the left or right side, potentially introducing bias. Therefore, no preference could be determined.

To determine the effect of age on swimming speed and swimming distance for coral and oyster larvae, one-way ANOVA's were performed. Age was used as a between groups variable, as for each age a different batch (from one larval population) was used. All data were found to be normally distributed (Shapiro-Wilk, $p > 0.050$) with homogeneity of variances (Levene's test, $p > 0.050$) after a log10 transformation. A Bonferroni post-hoc was performed to follow up a significant effect. *t*-tests and ANOVA's were performed using SPSS 25.0 (IBM Corporation, USA). Values reported are means ± standard error of the mean (SEM), unless stated otherwise.

3. Results

Both coral and oyster larvae were tracked successfully in the choice chambers, resulting in tracking paths and heat maps (Fig. 2). Coral larvae mostly swam in a linear pattern, and occasionally moved in circles (Supplementary Video 1). They also swam along the edges of their lanes, which is reflected in the heat maps. When added to the lanes, oyster larvae dropped to the chamber floor and laid still for 5 to 10 min. Subsequently, larvae started swimming in vertical spirals in which they were restricted by the limited water height.

Mean swimming speed by coral larvae ranged from 0.31 ± 0.07 to 0.34 ± 0.04 mm s^{-1} (~1 m h^{-1}), and mean total distance travelled in 90 min from 1489 ± 233 to 1659 ± 229 mm (Fig. 3). Overall, this constituted a relative swimming speed of approximately 0.2 body lengths s^{-1}. Larval age did not significantly affect swimming speed or distance travelled (Supplementary Table 1). For oyster larvae, mean swimming speed varied from 0.03 ± 0.01 to 0.10 ± 0.03 mm s^{-1}, and mean distance travelled ranged from 128 ± 46 to 446 ± 126 mm (Fig. 3). Overall, this constituted a relative swimming speed of approximately 0.4 body lengths s^{-1}. Larval age significantly affected swimming speed (Supplementary Table 1), which was lower at 19 dpr as compared to 14 dpr (Bonferroni, $p = 0.038$).

Coral larvae were significantly attracted to CCA chips within 90 min (Fig. 2, Supplementary Table 2), with the fraction of time spent on the CCA side of the lanes ranging from 0.66 ± 0.05 to 0.73 ± 0.06 (Fig. 4). They tended to spend most time near to or in contact with CCA chips once they had detected the CCA. Attraction of oyster larvae to oyster shells and limestone within the 90-min incubation period was not apparent, although this could not be tested statistically (Fig. 2).

4. Discussion

The incubation chambers with high resolution cameras have proven to be an effective setup/approach to study swimming and attraction behaviour of small marine organisms such as coral and oyster larvae. Even flat oyster larvae as small as 0.2 mm can be tracked with the high resolution camera, at least when fed with algae to darken them. With the setup described here, it is possible to conduct several temperature controlled, short-term experiments in one day. When taking six swimming lanes into account and assuming four consecutive runs, 24 larvae can be tested per day. When several cameras and chambers would be applied in parallel, high-throughput experiments could be carried out with animals from the same batch and thus age. This allows for multifactorial experiments during which several substrate types are tested, under a variety of environmental conditions, such as light colour or intensity, temperature, eutrophication or the effect of sounds. In addition to various materials and structures, chemical cues can be added to the lanes to quickly determine positive or negative effects on attraction. Ultimately, such experiments can help to systematically identify potential causes and solutions to reef recruitment bottlenecks. One potential application is the development of "flypaper" substrates which lure desired larvae to settle at desired locations. Tracking coral larvae enables quantification of attraction in terms of swimming speed and time until the larvae adhere to a certain substrate; the faster the attraction, the higher the chances that larvae seek refuge from ocean currents and subsequently settle at the desired location. In addition, deterrence of unwanted biofouling species such as barnacles, hydrozoans and annelids could be studied, which represent unwanted growth on ship hulls, monopiles of wind turbines and other artificial structures (Gollasch, 2002; Kerckhof, 2010). This could pave the way for the development of non-toxic anti-fouling coatings. For some species, the choice chambers described here may need to be customized depending on their size and swimming behaviour (see below).

The *F. fragum* larvae used in our study were quickly (within 90 min) attracted to the CCA chips provided and exhibited settlement behaviour, as larvae made physical contact with the chips. This attraction is in

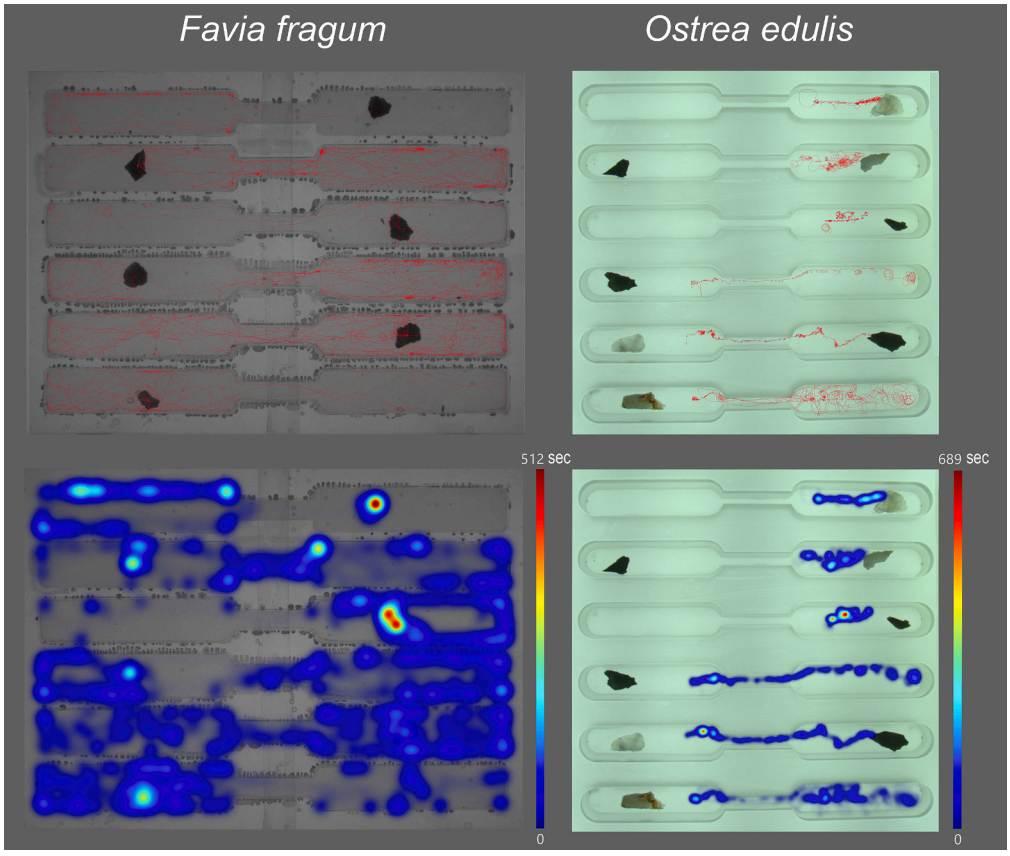

Fig. 2. Simultaneous activity tracking of 6 *Favia fragum* (left column) or 6 *Ostrea edulis* (right column) larvae for 90 min. The red tracks in the top row shows paths travelled by individual larvae, the bottom row shows the resulting heat maps. For *F. fragum*, the black chips in the lanes are crustose coralline algae. For *O. edulis*, fragments of limestone (black) and oyster shells (brown/white) are shown. The colour coding right to the heat maps indicates time spent at a given location in seconds (sec). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

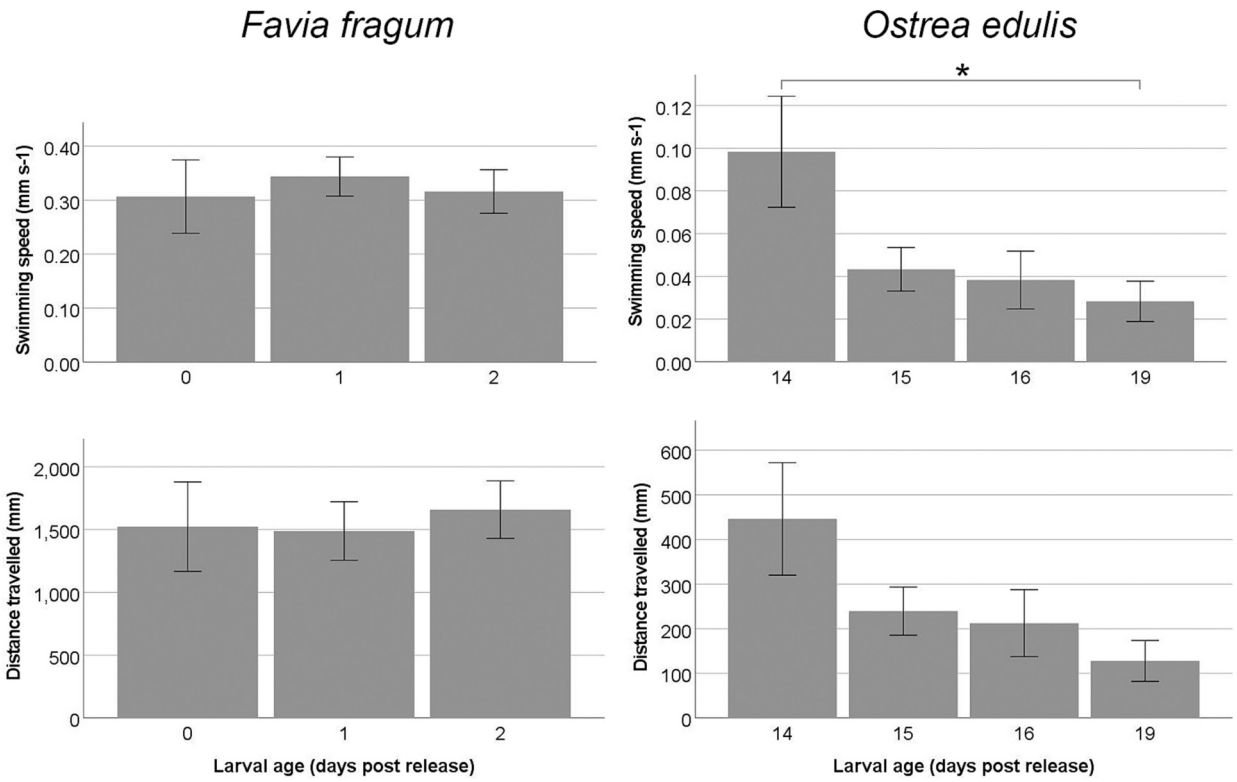

Fig. 3. Swimming speed and distance travelled of *Favia fragum* (left colum) and *Ostrea edulis* (right column) larvae measured during 90 min. Stationary larvae were excluded from swimming speed data. Larval ages are days post release (dpr) from the parent animal. The asterisk denotes a significant difference in swimming speed (Bonferroni, $p = 0.038$). Values are mean ± SEM with $N = 18$ larvae per time point for *F. fragum* and and $N = 6$ for *O. edulis*.

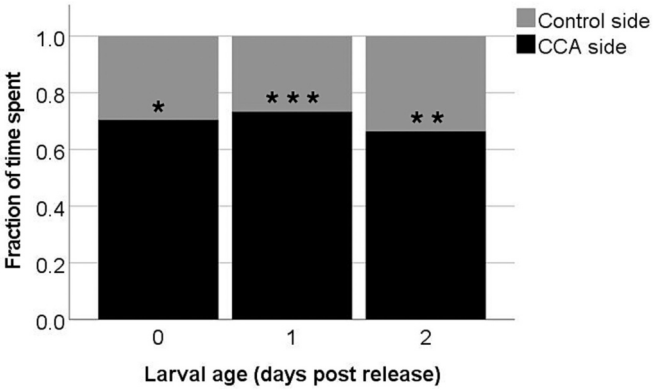

Fig. 4. Fraction of time *F. fragum* larvae spent on the side containing a CCA (crustose coralline algae) fragment or on the empty control side of the choice chamber after 90 min (N = 18 larvae per time point). Asterisks indicate significant differences between fractions: $*p < 0.050$, $**p < 0.010$, $***p < 0.001$ (one-sample *t*-tests with 0.5 as reference value).

agreement with previous research, and could have been mediated by metabolites produced by the CCA as well as their epibiotic bacteria (Tebben et al., 2015; Gomez-Lemos et al., 2018; Jorissen et al., 2021; Petersen et al., 2021), although our design does not allow us to pinpoint what CCA attribute(s) played a role in our experiments. This attraction was apparent immediately after release by parent colonies and maintained across three consecutive days. Conversely, *O. edulis* larvae are older when they become settlement-competent (Rodriguez-Perez et al., 2020), congruent with the observed decline in swimming speed between 14 and 19 dpr.

The swimming speed of *F. fragum* larvae recorded in our study (~0.3 mm s^{-1}) was relatively low when compared to previous research (Hata et al., 2017; Sakai et al., 2020). Unlike in this previous research, we included stagnant periods in averaging over a 90-min interval to include (pre-)settlement behaviour. When excluding stagnant periods, *F. fragum* swimming speeds were comparable to previous research at 1–1.5 mm s^{-1}, or approximately 1 body lengths s^{-1}. The swimming speed of *O. edulis* larvae lies within the same range as previously reported for *O. edulis* (Rodriguez-Perez et al., 2020) and the related species *Crassostrea gigas* and *Mytilus edulis* (Troost et al., 2008). Although the oyster larvae did not show settlement behaviour, their recorded swimming speed was on the lower end due to interval averaging. Rodriguez-Perez et al. (2020) reported that horizontal swimming speed is lower than their vertical swimming speed. During our experiments, we noticed the oyster larvae attempted to swim vertically, and they also performed circular swimming as was reported by Rodriguez-Perez et al. (2020). Unfortunately, our setup was not yet suitable for tracking vertical and circular swimming, so the current observation chamber has to be adapted to be able to study their full swimming and pre-settlement behaviour. Future designs should allow the larvae to move up and down in a more natural manner, which requires a vertical chamber and lateral camera setup.

To our knowledge, nothing has been reported about swimming distances of coral larvae in the field, although Roepke et al. (2022) published comparable values in a lab setting using a similar approach when this paper was under review. Although it is known that coral larvae can travel tens to hundreds of kilometers on ocean currents (Willis and Oliver, 1990; Graham et al., 2008), the contribution of active swimming to dispersal has remained unclear. Based on our data, this contribution seems negligible when compared to reef currents, which are typically one to two orders of magnitude higher (Shashar et al., 1996; Hata et al., 2017). However, swimming behaviour is expected to be very important during microhabitat selection; when larvae approach a reef by swimming perpendicularly to the current, they enter the reef boundary layer where water flow rates are considerably lower and where eddies can

entrain and transport larvae to substrata (Shashar et al., 1996; Reidenbach et al., 2006; Koehl and Hadfield, 2010). This may involve sinking behaviour based on specific combinations of light intensity and spectrum, low water pressure and reef sounds (Vermeij et al., 2010; Gleason and Hofmann, 2011), followed by recognition of substrate biofilm, colour and microstructure during which larvae seek out sheltered crevices on the reef (Morse et al., 1994; Petersen et al., 2005; Birrell et al., 2008; Nozawa, 2008; Erwin and Szmant, 2010; Mason et al., 2011; Mesias-Gansbiller et al., 2013; Tebben et al., 2015; Whalan et al., 2015; Gomez-Lemos et al., 2018; Jorissen et al., 2021). Similarly, larval dispersal of flat oysters is expected to be mostly dependent on currents rather than swimming behaviour (Smyth et al., 2016; Rodriguez-Perez et al., 2020). Therefore, oyster larval swimming will likely be especially relevant in the microhabitat selection phase, where bivalve shells are a preferred settlement substrate (Christianen et al., 2018; Rodriguez-Perez et al., 2020).

5. Conclusions

Our improved high-resolution tracking system enables the study of marine larval behaviour, with applications in fundamental and applied marine animal ecology. Most notable will be the systematic identification of key settlement cues. This could form a basis for future development of "flypaper" substrates which lure larvae to settle at desired locations, or deterrents to discourage larvae of undesired fouling species from settling on specific structures.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2023.151910.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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