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Sediment macrofaunal response to the diel oxygen cycle

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ABSTRACT: Infauna exhibit a range of behavioral responses to declining dissolved oxygen concentrations that affect their burrowing and feeding behaviors. Diel oxygen cycles, common in shallow coastal areas, may drive changes in faunal behavior that affect sediment mixing. In this laboratory study we exposed 3 species, the burrowing ophiuroid *Hemipholis cordifera*, the tube-dwelling polychaete *Owenia fusiformis*, and the burrowing clam *Ameritella versicolor*, to 60 h of diel cycling dissolved oxygen in the overlying water, with oxygen concentration varying between 2 and 7 mg l^{-1}. We observed the study organisms' behaviors and evaluated their sediment mixing activity using luminophores applied to the sediment surface. We found that sediment mixing activity of all 3 taxa, measured as percent decrease in luminophore coverage, varied proportionally with dissolved oxygen concentration during the diel cycle. Observations of animal behavior did not reveal a diel pattern, though this was likely due to the temporal and spatial scale of observations. Our results also indicated that diel cycling oxygen may change faunal effects on the sediment in ways that only emerge after more than a single cycle. Measuring sediment mixing in sustained full oxygen saturation may produce misleading estimates over time, and future research should investigate how faunal responses to short-term variability can scale to have longer-term effects.

KEY WORDS: Behavior · Bioturbation · Diel cycle · Hypoxia · Macrofauna · Oxygen · Sediment

1. INTRODUCTION

Marine sediment infauna can change the physical and chemical structure of sediments. By burrowing through and feeding on sediments and irrigating their burrow or tube structures, macrofauna mix solids and solutes into the sediments, which stimulates the sediment microbial community and increases nutrient cycling and organic matter breakdown (Aller 1978, Mermillod-Blondin et al. 2004, Mermillod-Blondin & Rosenberg 2006, Meysman et al. 2006). In prolonged, severe low dissolved oxygen (hereafter DO or DO concentration) conditions, these processes can be diminished or lost as sediment fauna experience stress or mortality (Diaz & Rosenberg 1995, Middelburg & Levin 2009, Sturdivant et al. 2012). However, in shallow coastal waters, DO patterns often follow a diel cycle as DO concentration decreases at night due to respiration and then increases during the day with photosynthesis (Wenner et al. 2004, Tyler et al. 2009). Organisms in coastal sediments, particularly if they are in organic-enriched sediments within photosynthetic depths, may therefore be exposed to extreme variation in DO availability on daily or even hourly timescales. When DO concentration declines to the point of being stressful, but not lethal, sediment macrofauna will often alter their bioturbation and bioirrigation behaviors (Diaz & Rosenberg 1995, Riedel et al. 2014). Thus, diel cycling DO may induce recurrent macrofaunal behavioral changes that affect their sediment mixing activity, particularly during the nightly period of the cycle.

Faunal responses to stress can be diverse and complex. Early studies classified animals into 1 of 2 types,

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based on their strategy of dealing with low DO: oxyconformers, which adjust their oxygen consumption with decreasing DO, and oxyregulators, which keep their oxygen consumption independent of DO, to a certain level (Prosser 1973). These classifications have utility as broad descriptors; however, researchers have noted that they do not fully capture the gradations in response as DO concentration changes (Mangum & Winkle 1973, Prosser 1973). More recently, researchers have expanded upon the regulator versus conformer model to better describe complex metabolic responses to and tolerances of declining DO (Mueller & Seymour 2011, Cobbs & Alexander 2018, Seibel et al. 2021).

Direct observations of sediment infaunal species exposed to hypoxic conditions have shown a wide range of physiological and behavioral responses to decreasing and low DO, with effects often occurring in stages as oxygen declines (Vismann 1990, Rosenberg et al. 1991, Nilsson & Rosenberg 1994, Diaz & Rosenberg 1995, Modig & Ólafsson 1998, Weissberger et al. 2009, Riedel et al. 2014). For example, the tube-dwelling polychaete worm *Diopatra cuprea* (Bosc, 1802) actively irrigates its tube with regular rhythmic bursts of activity, and the frequency of irrigation may not necessarily change linearly with oxygen concentration, i.e. after an initial decrease in irrigation rates upon encountering lowered oxygen saturation, some individuals maintain or even increase their irrigation rate as oxygen declines further (Mangum et al. 1968, Dales et al. 1970). This suggests that, on the time scale fauna would be most often encountering low oxygen, the diel scale, there may be undescribed complexity and variability in faunal behavioral responses. Taxa may vary in the severity of their responses to nightly low oxygen and in the time necessary to recover. With this variation in behavior and activity, we may expect corresponding differences in faunally mediated sediment mixing, a process that heavily influences sediment metabolism rates.

In this study, we assessed how infaunal behavior and sediment mixing activity vary with the diel oxygen cycle. Specifically, our goal was to evaluate within the diel cycle whether infaunal response to low DO depends only on DO concentration, or whether infauna respond differently to low DO depending on their recent low DO exposure. If infaunal responses to low DO depend only on DO concentration then we might predict a 'proportional' response pattern (H_1), wherein the frequency of animal behaviors that mix sediments and sediment mixing rates decrease and increase in direct proportion with diel cycling DO. Alternatively, if infauna are affected by recent low DO exposure we predict either a 'gasp' response (H_2), wherein, to speed recovery, animals exhibit greater mixing activity and more frequent mixing behaviors when DO begins increasing from the nightly minimum relative to the period before the minimum, or a 'lag' response (H_3), wherein animals are slow to recover from the nightly DO minimum and have lower mixing rates and less frequent mixing behaviors compared to the period before the minimum.

We selected 3 infaunal taxa: a burrowing brittlestar (*Hemipholis cordifera* [Bosc, 1802]), a tube-dwelling polychaete (*Owenia fusiformis* Delle Chiaje, 1844), and a burrowing clam (*Ameritella versicolor* [De Kay, 1843]). These taxa were selected based on their local availability and because they are all deposit/suspension feeders; thus, their feeding behavior, e.g. time spent feeding, could be assessed for diel changes. Additionally, these taxa have different life histories, burrowing strategies, and physiologies that provided a range of attributes to gauge behavior and activity change, and sufficient information exists in the literature to allow for *a priori* predictions of behavioral shifts.

1.1. *Hemipholis cordifera* (family Ophiactidae)

Burrowing brittlestars live in excavated burrows and bury their oral discs several centimeters down, using some of their arms to anchor and extending others up out of the sediment to feed in the water column or sweep along the surface (Woodley 1975). The burrow is ventilated by an undulating motion in the upward-extended arms, and this activity has been shown to drive increased flux of oxygen into the sediment in the brittlestar *Amphiura filiformis* (Vopel et al. 2003). Diel variation in *A. filiformis* activity, evaluated as the number of arms visibly protruding from the sediment, has been shown to be driven by photoperiod, increasing at night presumably so the animals avoid daytime predation by sighted predators (Rosenberg & Lundberg 2004). However, brittlestars have not, to our knowledge, been studied for responses to diel variation in DO. In shallow water, DO and light typically covary, and increased brittlestar arm extension at night may be an irrigation response to declining oxygen. We used the number of arms extended and surface evidence of excavation as an indicator of activity, predicting that *H. cordifera* would increase the number of arms extended upwards to facilitate burrow ventila-

tion as DO declines, and decrease the number of arms extending upwards to use them for excavation as DO rises.

1.2. *Owenia fusiformis* (family Oweniidae)

The polychaete *O. fusiformis* constructs a shingled tube out of bits of shell and can actively alter the tube's position in the sediment (Fager 1964, Eckman et al. 1981, Noffke et al. 2009). It extends its tentacular crown out of the tube and up into the water to suspension feed or bends the anterior portion of the tube down to deposit feed on the surrounding sediment, creating a characteristic feeding pit (Dales 1957). *O. fusiformis* has demonstrated a high tolerance of sustained low oxygen by ceasing its activity (Dales 1958). We predicted that with declining DO *O. fusiformis* would extend its crown upwards (respiring/suspension feeding) a greater proportion of the time rather than feeding on surface sediments or retracting into its tube and would defecate less frequently. We also predicted that mixing activity, observed from the surface, would vary with DO.

1.3. *Ameritella versicolor* (family Tellinidae)

Burrowing clams are biodiffusive mixers, their activities resulting in random diffusive sediment transport as opposed to the directional transport of burrow- or tube-dwelling fauna (Michaud et al. 2005). Tellenid clams deposit feed at the sediment surface or in the overlying water using their extendable incurrent siphons, and eject filtered water and material from their excurrent siphons in bursts (Volkenborn et al. 2012). They have been shown to intermittently irrigate surrounding sediments while deposit feeding, inducing porewater pressurization that oxygenates subsurface sediments in pulses. Little is known about how the mixing or ventilation behaviors of clams change during low DO, though it is known that sustained hypoxia drives *Macoma balthica* to decrease its burial depth (Long et al. 2008). There is little existing information on the habits of our study species, *A. versicolor*; however, we expected its general feeding and burrowing behaviors to resemble other well studied tellenid taxa, and in particular that response to low oxygen would be detectable as changes in feeding and irrigation. We predicted *A. versicolor* to decrease feeding and mixing activity with falling DO and increase their activity as DO increases.

2. MATERIALS AND METHODS

2.1. Animal and sediment collection

Sediment and study animals were collected from Petit Bois Pass (30.231°, −88.373°), between Dauphin and Petit Bois islands, in Alabama, USA, on April 27, 2020. The site was at ~5 m depth and had a salinity of 25 psu at the time of collection. Data from the closest station (Katrina Cut) in the ARCOS monitoring network for the month surrounding the collection date revealed large variation in DO concentrations on daily timescales and oxygen minimums approaching and occasionally surpassing hypoxia (Fig. 1). After collection, the animals were brought to the laboratory and the fully intact and active animals selected. Those animals were temporarily held in 2.12 l Tupperware containers filled with sediment from the collection site, submerged in a 113.6 l tank of aerated seawater mixed with an aquarium pump at a salinity of 25 and temperature of 25°C. Animals were not fed while in the tank and were kept in the dark except during tank maintenance and biweekly water changes. In previous projects, sediment fauna maintained in this way have remained healthy and active for several weeks post-collection.

Sediment was hand-sieved to remove large infauna, then thoroughly homogenized in a kitchen blender to eradicate small infauna. The blended sediment was used to fill 16 microcosms, i.e. thin, transparent aquaria for viewing infauna from the side (internal dimensions, 10.2 × 10.2 × 1.2 cm). The sediment was allowed to settle for 2 d and then topped off with more blended sediment so that the sediment–water interface was roughly even with the top edge of the microcosm. Filled microcosms were placed in a large holding tank with seawater (25 psu) for 4 d and allowed to settle and develop visible redox layering. Then, animals were removed from the Tupperware containers and 1 individual potted into each of 4 replicate microcosms for each taxon. Four microcosms were left with only sediment for a control treatment. Microcosms were then returned to the bubbled holding tank and kept there until use in an experimental trial.

2.2. Exposure setup

The laboratory setup for the experiment is described in detail in Gadeken & Dorgan (2021). DO concentration was manipulated in a diel cycle in the laboratory using a custom-built Arduino-based con-

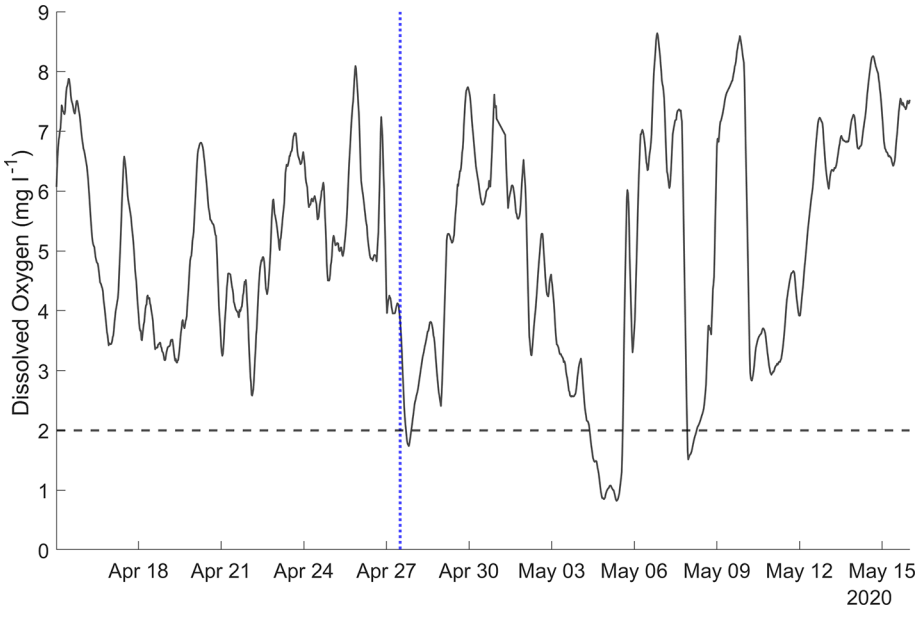

Fig. 1. Dissolved oxygen concentrations from the month surrounding animal collection. Data is from the Katrina Cut station of Alabama's Real-Time Coastal Observing System (ARCOS, https://arcos.disl.org/). Measurements were taken ~0.5 m off the bottom. Blue vertical line is the date of collection; dashed horizontal line the threshold for hypoxia

troller integrated into a closed loop aquarium system, hereafter called the 'oxygen manipulation machine' (OMM) (Fig. 2). An experimental tank (76 l clear aquarium) held the microcosms, and a layer of organic-rich mud at the bottom of the tank consumed oxygen. A sheet of oxygen-impermeable bubble wrap was placed on the surface of the experimental tank to prevent oxygen exchange with the air. When the DO in the tank dropped below a pre-programmed level, a valve opened and allowed oxygenated water to flow in from an upstream reservoir tank, adjusting the DO in the experimental tank through time to match the programmed pattern. Prior testing of the OMM showed that the system is capable of producing repeated diel oxygen cycles ranging from 3 to 7 mg l^{-1}, with ~89% of measurements deviating from the pre-programmed concentration by less than 0.23 mg l^{-1} (Gadeken & Dorgan 2021).

The experimental tank could not hold all the microcosms simultaneously and allow for visualization and photos of each tank, so the experiment was run in 4 replicate trials with 1 replicate of each animal treatment in the experimental tank for each trial. During each trial, the OMM was programmed to execute 2 full diel oxygen cycles, with 6 h of sustained high and low oxygen at the beginning and end of the trial, respectively (Fig. 3). These periods were included to provide baselines of animal behavior to which behavior during the diel cycles could be compared. This resulted in 3 × 12 h periods of declining DO and 2 × 12 h periods of increasing DO in each trial. All trials were begun late at night in real time so that the DO minimums of the diel cycles occurred in late afternoon. This was done so that the most sampling-intensive period of the trials occurred during daylight hours to ease data collection. The level of high DO was set as 7 mg l^{-1}, just under the calculated oxygen solubility concentration (7.17 mg l^{-1}) at the average experimental salinity (25) and temperature (25°C); the low DO concentration was set at the widely accepted threshold for hypoxia, 2 mg l^{-1} (Rosenberg et al. 1991). After each trial, the water in the experimental tank (~50% of the water in the entire system) was exchanged to prevent buildup of toxic metabolites, and a small amount of fresh mud was added to the sediment at the bottom to replenish the sediment organic matter and encourage DO consumption.

2.3. Sediment mixing

Changes in sediment mixing throughout the trial were assessed using luminophores — sediment particles covered in fluorescent paint (Solan et al. 2004, Maire et al. 2008, Dorgan et al. 2020). The luminophores were dry sieved through a 250 µm and then a 63 µm sieve, and particles retained on the smaller sieve (fine to very fine sand) were used in the experiment. Luminophores were soaked in a saltwater solution before use in the experiment to pre-wet the luminophore particle surfaces. A thin layer of luminophores ~0.5 mm thick was applied to the surface of the sediment in the microcosms and pictures taken at specific measurement points during each experimental trial (blue points in Fig. 3). To take pic-

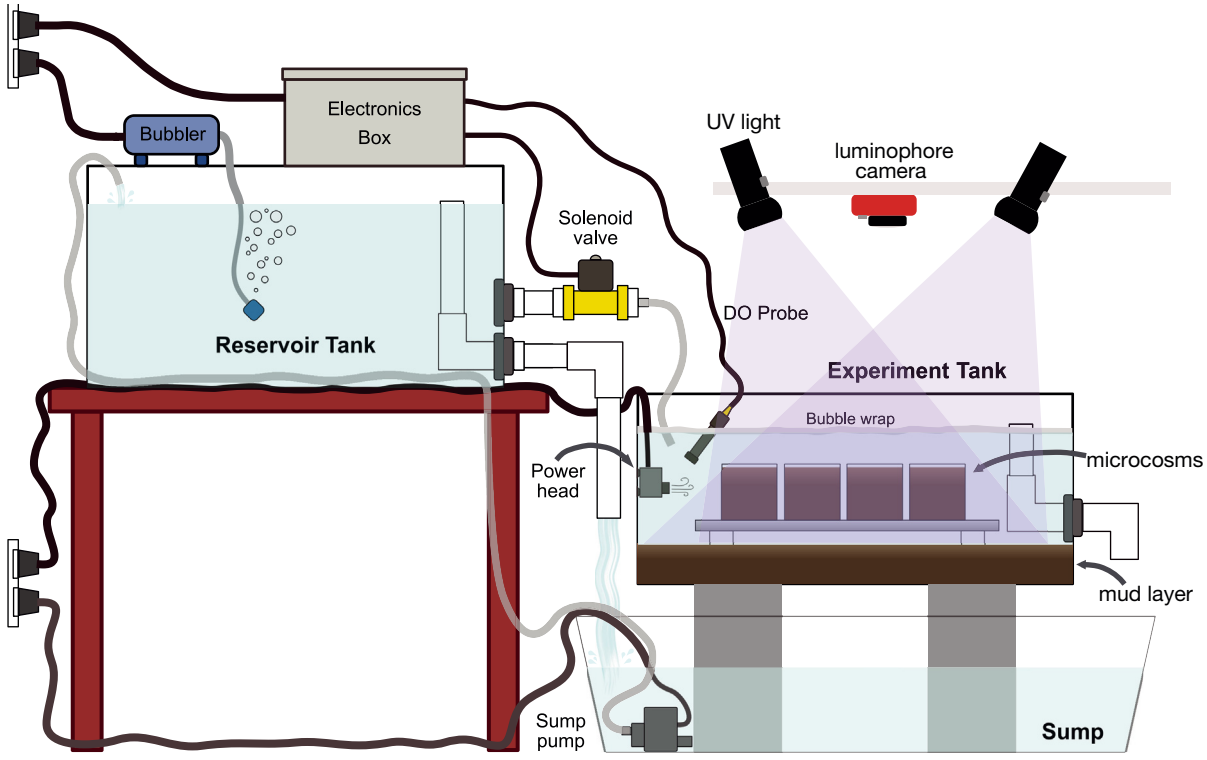

Fig. 2. Oxygen manipulation machine (OMM) schematic (after Fig. 1 in Gadeken & Dorgan 2021). The electronics box contained an Arduino microcontroller and an Atlas Scientific EZO DO circuit, connected to the dissolved oxygen (DO) probe in the experimental tank. A layer of organic-rich mud in the bottom of the experimental tank consumed DO. The Arduino was programmed to take periodic DO readings via the probe, and when DO was sensed to be below the pre-programmed level for that time, the solenoid valve was opened and oxygenated water from the reservoir tank allowed to flow in to increase the DO. Microcosms were placed on a raised platform in the experimental tank, and the water surface covered with bubble wrap to prevent oxygen exchange with the air. At measurement points throughout the trials, the UV lights were turned on and pictures were taken with a downward-facing luminophore camera mounted above the tank

tures, the bubble-wrap covering on the experimental tank was briefly pulled aside, the overhead room lights were turned off and UV lights shone at an angle onto the sediment surface to illuminate the luminophores. Pictures were taken using a horizontally mounted Olympus TG-2 Stylus camera with a top-down view of the microcosms.

Preliminary observations revealed that infauna can reduce coverage of luminophores from the sediment surface within a few hours, particularly in the areas

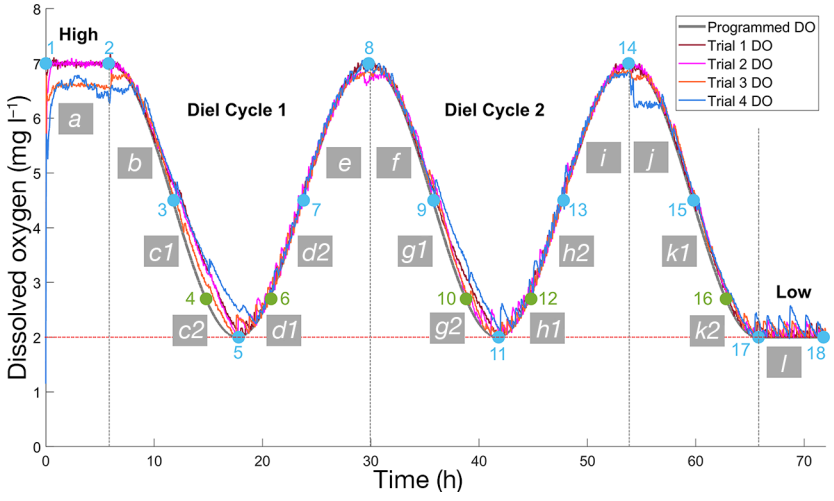

Fig. 3. Patterns of DO manipulation in the experimental trials. The OMM was programmed to execute 2 diel cycles with 6 h of high and low DO at the beginning and end of the trial, respectively. High DO concentration was set as 7 mg l^{-1} and low concentration as 2 mg l^{-1} (red dotted line). Blue circle points indicate when top-down pictures of luminophores were taken and new luminophores were applied. Green circles indicate when pictures were taken but luminophores were not applied. Numbers denote measurement points; lettered grey boxes denote measurement intervals. Vertical lines denote the 2 diel cycles (2–8 and 8–14) and the periods of high (1–2) and low (17–18) DO at the beginning and end

surrounding tube or burrow openings. To allow for repeated estimations of mixing activity throughout the multi-day trials, at the beginning of each measurement interval (grey lettered boxes in Fig. 3) additional luminophores were applied to areas of the sediment where most particles had been mixed down to re-cover the sediment, and an additional picture taken of the sediment surface. Then, initial pictures could be compared with final pictures for each interval to measure mixing activity during that interval (Fig. 4). At all mid-low points (Fig. 3, points 4, 6, 10, 12, and 16), pictures were taken but new luminophores not applied.

Measurement points were selected as the minimum, maximum, and midway points on the diel cycles as well as additional points just before and after the minimum DO. This resulted in 6 h measurement intervals in high DO periods of the diel cycle, and 3 h measurement intervals in the low DO periods. The extra points in the trough of the diel cycle (mid-low points) were included to increase temporal resolution and better capture possible 'gasp' or 'lag' responses as DO just began to increase from minimum. Mixing activity could, for example, be compared between interval *c2* and interval *d1* (Fig. 3) to determine if activity differs for the same DO concentrations when DO is falling vs. when it is rising.

Note that mixing activity is not a direct measure of bioturbation, which would require destructive sampling of sediments and preclude repeated measurement. It also alone cannot capture directionality of sediment mixing, as a sediment surface where luminophores have been subducted looks similar in top-down photos to a sediment surface where luminophores have been covered with sediment excavated from depth. However, it does serve as a useful proxy for mixing intensity and allows for repeated measures of activity for the same animal as DO varies. Further, based on what is known about the habits of these 3 taxa, it is reasonable to expect surface luminophore coverage to change with the intensity of their feeding and irrigating activities. A previous study that used luminophores to measure bioturbation in *Owenia fusiformis* and *Hemipholis elongata* (a congeneric of our brittle star *H. cordifera*) found that both taxa locally transported surface luminophores to depth, though via different methods (Dorgan et al. 2020). *O. fusiformis* created feeding pits around their tubes, clearing the luminophores from that area and redistributing them deeper, and *H. elongata* moved surface luminophores down into the animal's burrows, creating a subsurface luminophore maximum. Though luminophore mixing patterns have not been characterized specifically for *Ameritella versicolor*, given behaviors observed in other tellenid taxa it is likely that their activities will result in more diffusive mixing of luminophores across the entire microcosm surface rather than the localized transport occurring around *O. fusiformis* tubes or within *H. cordifera* burrows.

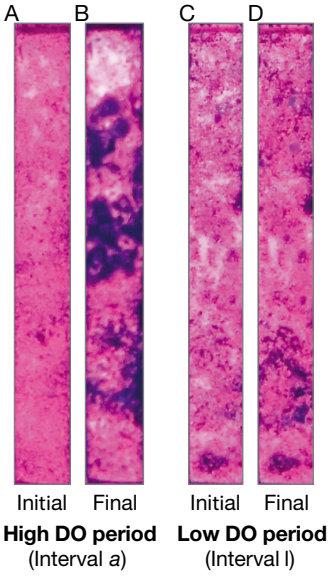

Fig. 4. Examples of top-down luminophore pictures analyzed for mixing activity. (A,B) Initial and final pictures taken of the microcosm in Trial 2 containing *Ameritella versicolor* in sustained high DO (interval *a* in Fig. 3); (C,D) initial and final pictures of the same microcosm in sustained low DO (interval *l* in Fig. 3)

Early in Trial 3 we noticed tracks in the surface sediments of the control treatment microcosm, and at measurement point 8 we extracted a small burrowing snail from the sediment. During data analysis we discarded the mixing activity measurements of that replicate from intervals before the snail was removed. Also, the flow control mechanism on the power head in the experimental tank (Fig. 2) malfunctioned at the beginning of Trial 3 and caused increased flow in the tank that appeared to remove luminophores from the top sediment layer of the microcosms. The flow control was repaired at measurement point 2 and mixing activity data from interval *a* of that trial was not used in the analysis (Fig. 3).

Luminophore pictures were analyzed using the image analysis software ImageJ (Rasband 2018). Images were cropped to contain only the microcosm sediment surface (Fig. 3), auto-contrasted to standardize brightness, converted to 8-bit, segmented using a brightness threshold to isolate the area covered by the luminophores (brightness: 70–255), and percent cover calculated. Mixing activity during each measurement interval in the trial was calculated as the difference between percent luminophore cover at the beginning and end of the interval. To test whether mixing activity in each of the animal treatments was greater than the control treatment, we

used Kruskal-Wallis tests with a Bonferroni correction for multiple comparisons. To test our 3 response hypotheses (proportional, gasp and lag), we compared mixing activity values from the declining and increasing portions of the diel cycle using pairwise Mann-Whitney *U*-tests. This was done for each taxon and within each of the 2 diel cycles in the exposure. The overall pattern of mixing activity throughout the exposure was assessed by fitting polynomial curves through the values for each animal treatment across trials, with DO concentration and the elapsed time in the trial as predictive variables. Analyses were performed in Matlab 2021a (https://mathworks.com).

2.4. Animal behavior

Animal behavior and activity were monitored with time-lapse photographs of the microcosms. GoPro Hero4 cameras with attached macro lenses were mounted outside the tank, positioned with a view at each of the microcosms containing animals through the tank wall. Room lights were kept on during all trials to allow for clear photography and to control for effects of lighting on behaviors. The cameras were programmed to take time-lapse photos once per minute for the duration of each trial and were compiled into a video for further analysis. Video segments were excluded at each measurement point because the lights were turned off to take photos of luminophores, and a portion of the video of the *O. fusiformis* in Trial 3 (amounting to ~5 hrs of real time) was lost due to camera malfunction.

Animal behavior was quantified using the open-source event-logging software BORIS (Friard & Gamba 2016). An ethogram of animal behaviors was constructed for each taxon (Table 1), and videos were examined frame-by-frame and coded for presence or absence of ethogram behaviors. For analysis, the event logs of all coded videos were exported from BORIS as binary tables binned in 3-minute (real-time) intervals. We calculated the proportion of time each animal spent performing the different behaviors for each 6 h interval of the exposure (blue measurement points in Fig. 3) and performed a Kruskal-Wallis test with a Bonferroni correction to compare these values between all the intervals and determine if behavior changed between when DO was falling and rising in the diel cycle.

3. RESULTS

The DO in the experimental tank followed the programmed pattern in all 4 trials (Fig. 3). In Trial 4, DO declined slightly more slowly than was necessary to keep pace with the programmed pattern in the trough of the first diel cycle, likely because the layer of mud consuming DO in the tank was becoming depleted in organic matter. However, DO decreased to 2.25 mg l^{-1} at the lowest point of the first cycle and tracked closer to the pre-programmed pattern in the second cycle.

3.1. Sediment mixing

Most mixing activity values were negative or close to zero, indicating that percent luminophore coverage either decreased or did not change (Fig. 5). Sediment mixing in the control treatments remained low and stable for the duration of the trial (Fig. 5A).

Reduction in luminophore coverage in all 3 animal treatments was highest and most variable in the high DO period at the beginning of each trial, generally decreasing in variability as the trials went on. No animal treatments differed in mixing activity from the control treatment in the sustained high DO period (interval *a*), though this was likely due to the small sample size from excluding data from Trial 3

Table 1. Behaviors used for ethography. Behaviors marked with a (*) are point events, all others are state events

Taxon	Behavior	Description
Hemipholis cordifera	Excavation	Sediment from depth is deposited on the sediment surface
	Arms extended (1–5)	Number of arms extended out of sediment
Owenia fusiformis	Crown retracted/not visible	Worm crown retracted into its tube, or the crown can't be seen
	Crown up	Respiring and/or suspension feeding (crown extended upwards)
	Crown down	Deposit feeding (crown repeatedly bent down to sediment surface)
	Defecation*	Production of fecal pellet at sediment surface
Ameritella versicolor	Irrigating/ventilating	Sediment is visibly pulsing upwards from clam ventilation
	Change location*	Focal point of pulsing sediment shifts elsewhere in the microcosm
	Feeding	Siphon(s) are visible probing through sediment

(Kruskal-Wallis with Bonferroni correction, $p > 0.05$). During the period at the end of the trials (interval *l*) animals appeared to be doing very little mixing, though *Ameritella versicolor* had significantly greater mixing rates than the control in that interval (Kruskal-Wallis with Bonferroni correction, $p = 0.03$).

In the first diel cycle *Hemipholis cordifera* showed significantly greater mixing just after the DO minimum than before (intervals *c2* and *d1*, Mann-Whitney Wilcoxon, $p = 0.03$), though this was not the case in the second diel cycle (intervals *g2* and *h1*, Mann-Whitney Wilcoxon, $p = 0.34$) (Fig. 5B). There was no difference between mixing activities in *H. cordifera* in the declining and rising mid-low intervals in either cycle (intervals *c1* and *d2* (Mann-Whitney Wilcoxon, $p = 0.86$), and intervals *g1* and *h2* (Mann-Whitney Wilcoxon, $p = 0.34$)). For both *Owenia fusiformis* and *A. versicolor* there were no differences in the mixing activities before and after the DO minimums, nor were there differences between the mid-low intervals in either diel cycle (Mann-Whitney Wilcoxon, all $p > 0.1$) (Fig. 5C,D).

All 3 animal treatments displayed a similar pattern of decreased mixing throughout the trial, punctuated by variation with the diel cycles. A polynomial curve was fit to the data for each taxon:

$$L = ax + bt^c + d \quad (1)$$

where L is percent luminophore coverage (i.e. mixing rate), x is DO concentration, t is the time in the trial, and a, b, c, and d are the term coefficients. In this equation, the first term (ax) is the variation due

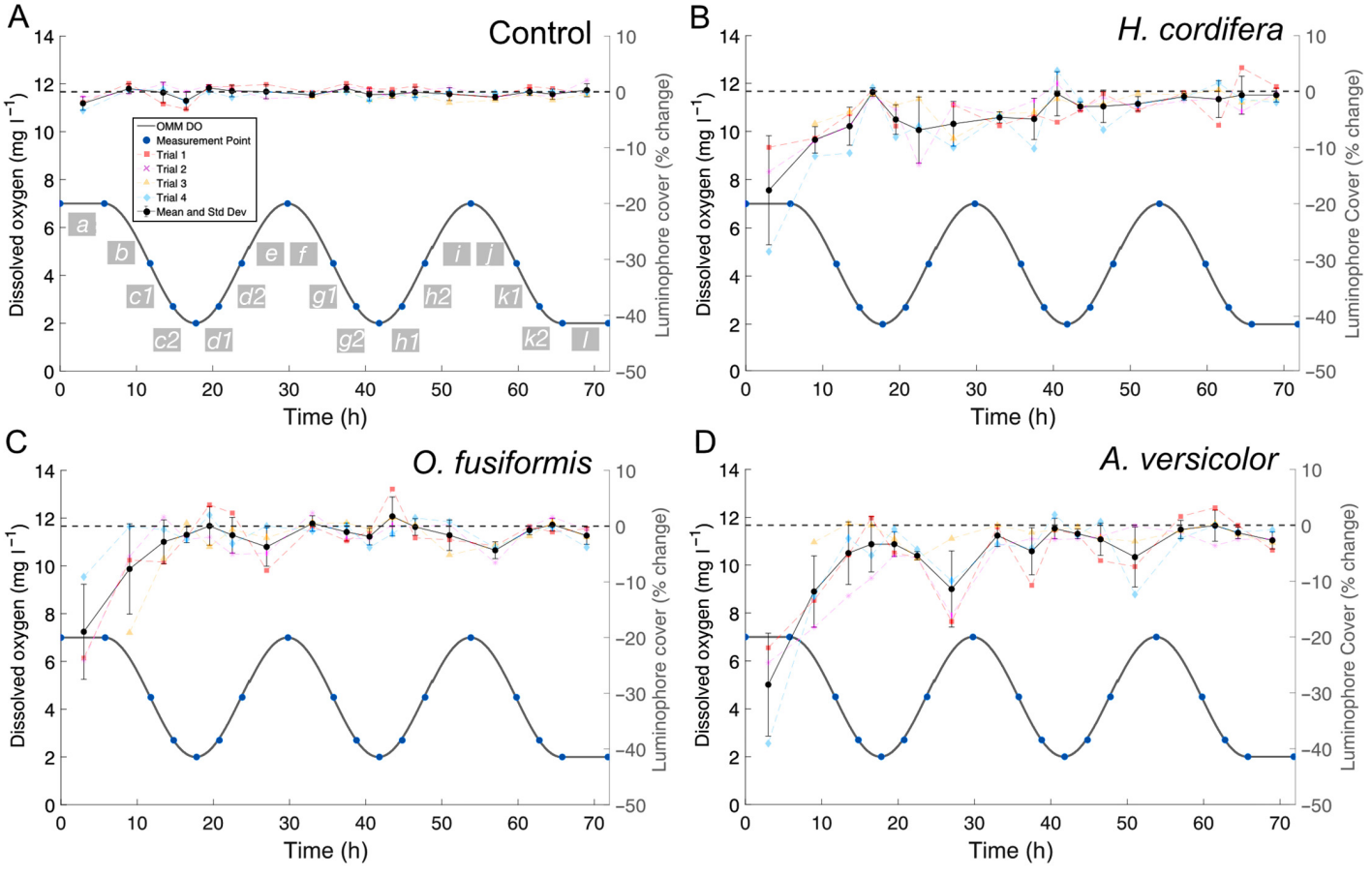

Fig. 5. Sediment mixing activity (right y-axis) for (A) Control, (B) *Hemipholis cordifera* (brittlestar), (C) *Owenia fusiformis* (polychaete worm), and (D) *Ameritella versicolor* (clam), throughout the trials, expressed as the change in percent of the sediment surface covered by luminophores in the microcosm throughout each interval (i.e. a negative value indicates luminophore cover had decreased over that interval). The dashed horizontal line at zero (right y-axis) demarcates the level at which there was no net change in luminophore cover. The average percentages and their standard deviations for each interval are plotted in black, with the data points for individual replicates in each trial shadowed behind them. The lettered grey boxes denoting the intervals have been included on (A) for ease of reference with the text, and the DO pattern programmed into the OMM and measurement points plotted on each to compare mixing activity throughout the trials (left *y*-axis). Blue dots on DO time-series show when pictures of luminophores were taken

to DO concentration, and the second term (bt^c) expresses the overall decrease in mixing throughout the entire time of the trial. The values for each term coefficient and their 95% confidence intervals were calculated for the animal treatments and the control. The coefficients, and therefore the terms in the function, were deemed statistically significant if the confidence interval did not include zero. Change in the control treatment mixing rate throughout the trial was not well explained by the function (no significant coefficients), while mixing rates in *O. fusiformis* and *A. versicolor* were significantly explained by both DO concentration (coefficient *a*) and the time in the trial (coefficients *b* and *c*) (Table 2). *H. cordifera* mixing rates were significantly explained by time in the trial, but not DO concentration (Table 2).

3.2. Animal behavior

3.2.1. *Hemipholis cordifera*

In 3 of the *H. cordifera* replicates the subsurface view of the animal through the microcosm wall was mostly obscured by sediment; however, the individual in Trial 1 was completely visible, allowing for direct observation of its below-ground activities and description of behaviors. The animal maintained a large, excavated space at depth for positioning its anchoring arms and oral disc, and pink luminophore particles could be seen at depth in the burrow and lining the burrow walls, indicating subduction or collapse of sediments from the surface (Fig. 6A). During burrow excavation, the animal transported sediment with its tube feet, conveying sediment particles up the anchoring arm, around its oral disc and up an extended arm to deposit it in a pile at the surface (Video S1 in the Supplement at www.int-res.com/articles/suppl/m703p067_supp/). Frequently, these sediments were deposited on top of the luminophore particles layered on the surface. The animal also occasionally reorientated its body by rotating its oral disc and shifting its arms, often moving an upwardly extended arm down to anchor or an anchoring arm upwards into the water column (Video S2). Top-down luminophore images showed absence of surface luminophores in the area surrounding *H. cordifera* burrow openings where its arms protruded (Fig. 6B).

All *H. cordifera* buried their central discs in the microcosms and remained buried throughout their experimental trials, extending their arms up into the water column and occasionally excavating sediment into piles around their arm holes. There was no difference in amount of time spent excavating between any of the time intervals throughout the trial, nor were there significant differences in the number of arms extended into the water column (Kruskal-Wallis with Bonferroni correction, $p > 0.05$) (Fig. 7A).

3.2.2. *Owenia fusiformis*

The crowns of all *O. fusiformis* individuals could be observed in the time-lapse videos (Fig. 6C). We observed the worms bending their crowns down to surface sediments to deposit feed around their tubes (Video S3), clearing the luminophores from that area and creating the characteristic feeding pit (Fig. 6D). Frequently, retraction of the crown into the worm's tube was followed by defecation (Video S4), and worm fecal pellets were observed to contain pink luminophore particles. Viewed through the microcosm wall, the volume of sediment surrounding the buried portion of the *O. fusiformis* tubes appeared to be oxygenated. We also observed several instances of worms rapidly moving upwards, partially unearthing their tubes and extending their crowns further up in the water column (Video S5).

There was considerable variability between *O. fusiformis* individuals in the amount of time spent performing the different behaviors and in the timing of behaviors throughout the exposure (Fig. 7B). We found no significant differences in the proportion of

Table 2. Values and confidence intervals of function ($L = ax + bt^c + d$) coefficients describing mixing activity. Asterisks indicate significant coefficients (the confidence interval does not include zero)

Taxon	*a*	*b*	*c*	*d*
Control	−0.03 ± 0.17	$-6.12e^3 \pm 1.85e^7$	$-7.49 \pm 2.76e^3$	−0.06 ± 0.68
Hemipholis cordifera	−0.47 ± 0.52	−31.67 ± 13.57*	−0.54 ± 0.53*	3.40 ± 7.64
Owenia fusiformis	−0.57 ± 0.51*	−63.16 ± 49.70*	−1.20 ± 0.70*	1.78 ± 2.62
Ameritella versicolor	−0.81 ± 0.64*	−70.33 ± 38.36*	−0.94 ± 0.49*	2.26 ± 4.15

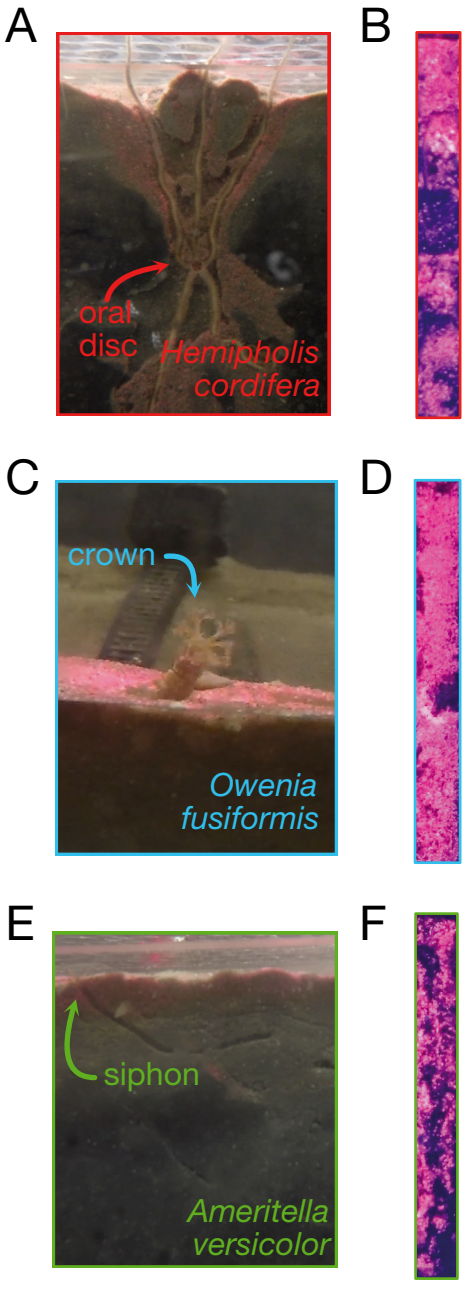

Fig. 6. (A,C,E) Side-view sample images of animals in microcosms and (B,D,F) top-down photos of microcosm surface with luminophores at measurement point 2 in Trial 4 for the 3 taxa (i.e. after the high oxygen portion of the exposure). *Hemipholis cordifera* anchors its body with some of its arms while stretching the others up into the water column to feed. In Trial 1, the entire animal was visible through the side of the microcosm (A). Pink luminophore particles could be seen at depth, indicating surface sediments had been subducted or collapsed into the burrow. *H.* cordifera excavates sediment from deep in the burrow and deposits it at the sediment surface, covering surface luminophores (B). *Owenia fusiformis* extends its crown up into the water column to suspension feed or respire and repeatedly bends its crown down to the surface to deposit feed (C). This clears the surrounding sediment of surface luminophores (D) and creates the characteristic feeding pit. *Ameritella versicolor* tunnels its extendable siphon through the sediment to feed, though the shell is not usually visible (E). Patchiness in luminophore coverage (F) indicates the clam surface deposit feeds over much of the microcosm area

time spent deposit feeding or suspension feeding throughout the trials (Kruskal-Wallis with Bonferroni correction, $p > 0.05$).

3.2.3. *Ameritella versicolor*

Though the clam's shells were buried and were not visible most of the time, we could easily observe them probing through the sediment with their siphons (Fig. 6E) (Video S6). There was also a notable pulsing motion occurring in the top layer of sediment, which we interpreted as the clam ventilating and ejecting water from its excurrent siphon, and in some trials the focal point of this pulsing would relocate elsewhere in the microcosm (Video S7). In the side view of the microcosms in the time-lapse videos, luminophore particles could be seen mixed down from the sediment surface; top-down images showed *A.versicolor* deposit feeding, which resulted in patchy coverage of luminophores across the microcosm sediment surface (Fig. 6F). In Trials 1, 2 and 3, irrigation was consistently observed throughout the entire trial duration, and in Trials 2 and 4, deposit feeding was consistently observed (Fig. 7C). There were no statistical differences between irrigation and feeding activity at the different measurement intervals throughout the trials (Kruskal-Wallis with Bonferroni correction, $p > 0.05$).

4. DISCUSSION

The diel variation and similarity of mixing activity in falling versus rising DO concentrations in *Owenia fusiformis* and *Ameritella versicolor* microcosms indicate that these taxa exhibited a proportional response (H_1) to the diel cycle. *Hemipholis cordifera* did exhibit greater mixing after the DO minimum in the first diel cycle, which indicates a gasp response (H_2); however, this response was diminished in the second diel cycle as overall mixing rates trended towards zero. More generally, sediment mixing declined and stabilized throughout each trial, decreasing from the high and variable levels in the

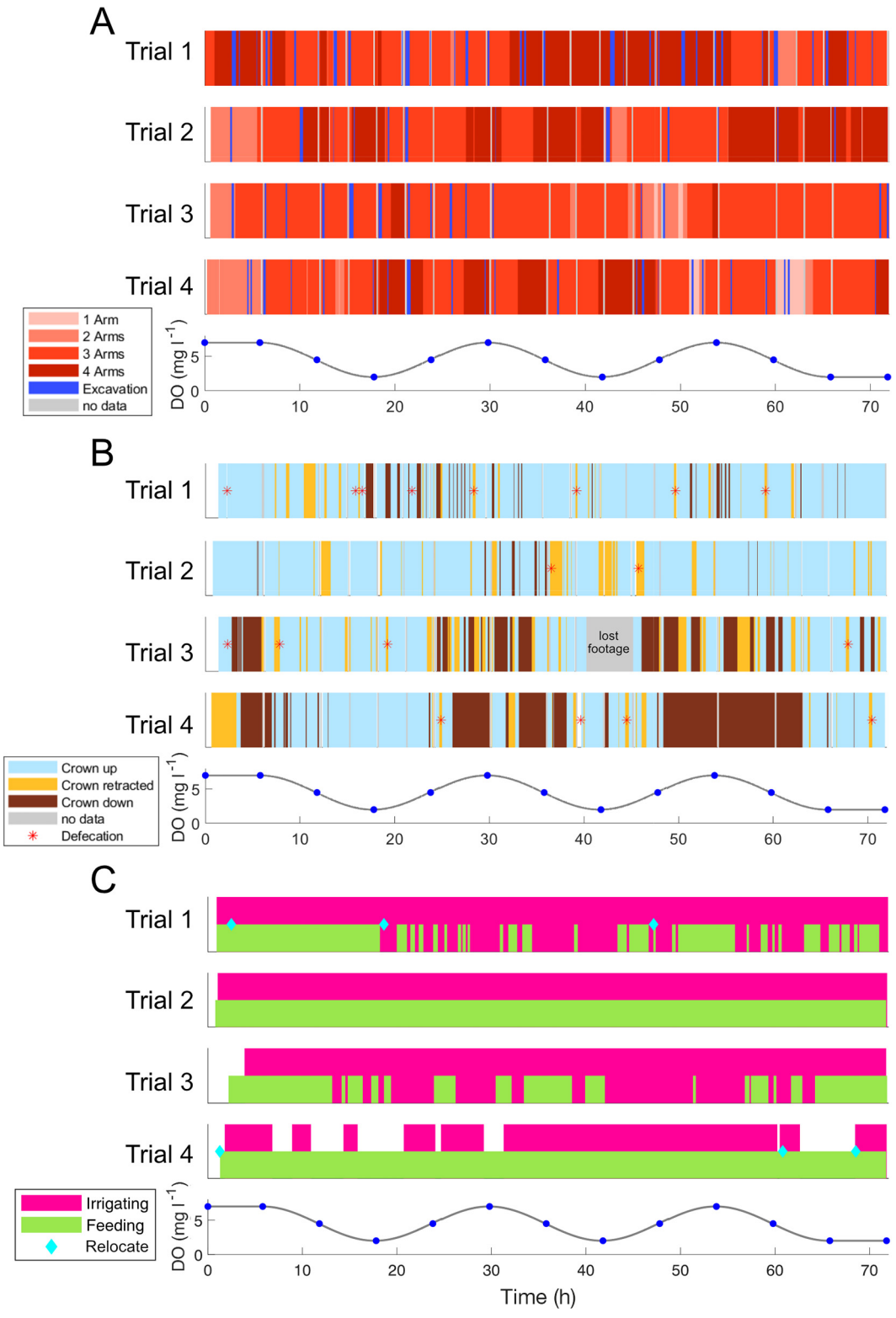

Fig. 7. Coded behavior patterns throughout the 4 trials for each of the 3 study taxa. (A) Variation in the number of *Hemipholis cordifera* arms elevated above the sediment and the timing of excavation events. (B) Times when *Owenia fusiformis* has its crown retracted into its tube versus extended up as well as when the extended crown was bent down to the sediment surface, and the frequency and timing of defecation. (C) A pulsing motion can be observed in surface sediments as *Ameritella versicolor* ejects water from its excurrent siphon. The location of this pulsing occasionally changed during the trial for some individuals

high DO interval at the beginning and approaching zero in the declining and low DO conditions at the end. This indicates that not only were the animals responding to changing DO but were mixing sediments less intensely than what would be expected in sustained high or saturated DO conditions. It is also notable that for all 3 animal species, mixing activity declined exponentially throughout the trial and over the 2 replicate diel oxygen cycle exposures, and mixing activity for *H. cordifera* showed no variation with the diel oxygen cycle.

The experiment was designed to evaluate differences in faunal behaviors and sediment mixing within diel cycles rather than across cycles, and therefore did not include control sustained high and low DO exposures to compare mixing activity. However, the significant polynomial fits of the data show that mixing activity was driven both by diel oxygen variation and by the time elapsed in the exposure, which indicates that for these animals repeat exposure to the diel oxygen cycle overall drove mixing activity down, dampening it even at points in the cycle when DO was high. This raises an intriguing question of whether further repeated exposure to diel oxygen variation would continue to drive mixing activity down or if instead it would stabilize, with a diel-driven proportional response still occurring, albeit at a diminished magnitude.

The combination of the top-down luminophore photos and the side-view time-lapse videos allowed us to characterize the different methods of particle displacement of the 3 taxa. For *H. cordifera*, the combination of downward collapse and upward excavation of sediments likely resulted in active and deep vertical mixing as well as apparently extensive oxygenation of the burrow cavity and lining burrow walls (Video S1). However, surface sediment disruption was somewhat constrained horizontally to the area around the burrow openings (Fig. 6B), which did not change location for any individuals during the trials. This contrasts with *A. versicolor*, which mixed luminophores more shallowly and diffusely, doing so over much of the microcosm surface by extensively probing with its long, extendable siphon to feed (Fig. 6F, Video S6). *O. fusiformis* fed on sediments in a limited area around its tube (Fig. 6D, Video S3); however, unlike either of the other studied taxa, it subsequently consolidated sediments at the surface by defecation (Video S4). Visual inspection of the top-down luminophore images throughout the trials indicated that the overall decrease in mixing activity was driven by decreases in these mixing behaviors specific to each taxon.

Given the apparent change in mixing activity throughout the trials, it was surprising that no corresponding behavioral patterns were observed for any of the taxa (Fig. 7). Behavior patterns were highly variable between replicates within taxa, which may have obscured the effect of changing DO concentration. However, it is more likely that faunal behaviors that removed particles from the sediment surface were not captured by our time-lapse imaging. In both *H. cordifera* and *O. fusiformis*, for example, it would be difficult to tell through image analysis whether the animals are suspension feeding or not, as suspension feeding and respiration occur in similar postures. Additionally, short duration or point-event behaviors that directly related to mixing activity variation (e.g. sediment excavation by *H. cordifera*, defecation by *O. fusiformis*) likely were not captured at a high enough frequency within each measurement interval to statistically detect a relationship with oxygen concentration. The pulsing driven by ejection of water from the excurrent siphon and the deposit feeding behaviors observed in *A. versicolor* are consistent with behavior observed in other tellenid species (Volkenborn et al. 2012). However, ventilation and deposit feeding were consistently observed among 3 of the 4 replicates and did not cease as DO decreased, (Fig. 7C), despite mixing activity decreasing throughout the trials, which indicates that DO variation may not determine whether the clams ventilate and feed but rather the frequency and amplitude of ventilation and the amount of feeding.

5. CONCLUSIONS

Most laboratory studies on the mixing of sediments by fauna make no mention of the DO concentrations in their experimental setups, but presumably DO was maintained at high or saturated levels for the duration of the experiments (Pelegri & Blackburn 1995, Widdicombe & Austen 1999, Mermillod-Blondin et al. 2004, Michaud et al. 2005). Those studies that focus on faunal responses to low oxygen typically expose the animals to sustained conditions of different treatment DO concentrations (e.g. high vs. low) (Seitz et al. 2003, Weissberger et al. 2009, Calder-Potts et al. 2015), or if variable DO is included, it is as an exposure in unidirectional declining oxygen (Dales et al. 1970, Kristensen 1983, Riedel et al. 2014), equivalent to taking measurements up to the minimum DO of the first diel cycle in our trial pattern. Our results indicate that measurements of sediment mixing by fauna in sustained high DO may pro-

duce overestimations of long-term mixing rates, particularly for animals that in *in situ* conditions would be experiencing variable DO. Additionally, it may require more than a single exposure to declining DO concentration, even more than a full diel cycle, for behaviors to emerge and stabilize into a repeating pattern that may be representative of *in situ* responses. Short-term variability and the responses it induces can scale to have longer-term effects, and a better description of this linkage is critical to improving both conceptual and numerical models of dynamic coastal systems.

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