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Research Article

Larval Diel Vertical Migration of the Marine Gastropod *Kelletia kelletii* (Forbes, 1850)

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Documenting larval behavior is critical for building an understanding of larval dispersal dynamics and resultant population connectivity. Nocturnal diel vertical migration (DVM), a daily migration towards the surface of the water column at night and downward during the day, can profoundly influence dispersal outcomes. Via laboratory experiments we investigated whether marine gastropod *Kelletia kelletii* larvae undergo nocturnal DVM and whether the behavior was influenced by the presence of light, ontogeny, and laboratory culturing column height. Larvae exhibited a daily migration pattern consistent with nocturnal diel vertical migration with lower average vertical positioning (ZCM) during day-time hours and higher vertical positioning at night-time hours. ZCM patterns varied throughout ontogeny; larvae became more demersal as they approached competency. There was no effect of column height on larval ZCM. DVM behavior persisted in the absence of light, indicating a possible endogenous rhythm. Findings from field plankton tows corroborated laboratory nocturnal DVM findings; significantly more *K. kelletii* were found in surface waters at midnight compared to at noon. Unraveling the timing of and the cues initiating DVM behavior in *K. kelletii* larvae can help build predictive models of dispersal outcomes for this emerging fishery species.

1. Introduction

In open coast marine habitats, multiple factors influence larval dispersal destinations, including abiotic factors, such as current speed and direction, and biotic factors, such as timing of larval release and pelagic larval duration (PLD). Additionally, larvae of marine species living within estuaries, on coastal shelves, or near oceanic islands have been hypothesized to use vertical positioning behaviors that, when coupled with stratified countercurrents, promote retention or return to suitable settlement habitat [1–3]. Documenting this form of larval behavior is a critical component to building an understanding of larval dispersal dynamics [3–6].

Diel vertical migration (DVM) has been implicated in both modeling [7, 8] and empirical studies [9] as a positioning behavior that can affect dispersal outcomes. In nocturnal DVM, larvae migrate toward the surface of the water column at night, and then downward in the water column during the day. Reverse DVM shows the opposite pattern, but it is also thought to influence dispersal outcomes (e.g., [10]). These vertical migratory behaviors, or sensory capabilities that are consistent with vertical movement behaviors [11], have been observed in a wide variety of taxa, including arthropods [12], mollusks [10, 13–15], fish [16], and others [17].

Diel vertical migration is primarily controlled by light, with other factors acting as modifiers of this behavior [18, 19]. Light plays a multifaceted role in DVM because it may (1) signal organisms to start or stop swimming (i.e., photokinesis) [20], (2) provide cues for the direction of swimming (i.e., positive or negative phototaxis) [21], and (3) entrain endogenous rhythms so that behavior persists in the absence of light [22]. In addition to light, other cues such as gravity,

temperature, oxygen, salinity, pressure, and chemicals from phytoplankton and predators may influence DVM [18, 23–26].

While light may initiate, signal the direction of, and entrain DVM behavior, the behavior itself varies throughout ontogeny. This variation may be associated with size-dependent predation risk where younger, smaller individuals alter their DVM patterns in the presence of predators, while older, larger individuals, who are less vulnerable, do not alter their behavior [27]. Alternately, younger oyster (*Crassostrea virginica*) larvae remain evenly distributed throughout the water column while older larvae rise during the flood tide and sink during the ebb tide [28]. The latter behavior enhances retention within an estuary, enhances up-estuary transport, and provides opportunity for the larvae to sample the substrate for suitable habitat. A similar pattern has been observed in scallop larvae, *Placopecten magellanicus* [13], in the open sea and blue crab megalopae, *Callinectes sapidus* [29], in estuaries. Thus, complex larval positioning behaviors that vary across a day or through ontogeny or both can impact how and where larvae disperse [3].

There is growing interest in the dispersal dynamics of Kellet's whelk, *Kelletia kelletii*, a large predatory neogastropod inhabiting rocky reefs and kelp forests along the coast of California, USA, and Baja, Mexico. *Kelletia kelletii* is slow-growing, slow to mature, and aggregates seasonally for mating; these traits make this recently targeted fishery species vulnerable to overexploitation. The *K. kelletii* fishery has experienced a rapid increase in landings since 1995 [30], prompting the California Department of Fish and Game to designate the species as an "emerging fishery" (CA Regulatory Notice Register 2011 43-Z; Craig Shuman, personal communication). This species has a pelagic larval duration (PLD) of at least 5.5 weeks (M. Romero and D. Zacherl, unpublished data), making long-distance dispersal a possibility, though even species with long PLD are capable of retention or very short-distance dispersal [31]. Recent molecular work on *K. kelletii* based on microsatellite markers suggests broad exchange of larvae among populations (i.e., global F_{ST} = 0.00138) [32]. However, such broad exchange might be occurring over temporal scales of decades to centuries [33] and may not reflect year-to-year exchange likely to be more relevant for fishery management. Knowledge of the larval behavior of this species would facilitate development of oceanographic models of dispersal that incorporate this behavior, against which the molecular results could be compared.

Using a series of controlled laboratory experiments and field plankton tows, we explored whether *Kelletia kelletii* larvae exhibit nocturnal DVM and what factors (including light, ontogeny, and culture column artifacts) influence their DVM behavior. The following specific research questions were addressed. (1) Do larvae exhibit nocturnal DVM in laboratory cultures and, if so, does the pattern of DVM change as a function of ontogeny? (2) Does light cue DVM ascent or descent behaviors? (3) Does culturing column height affect DVM behavior? (4) Do larvae in the field exhibit distributions that are consistent with nocturnal DVM behavior?

2. Methods

2.1. Study Organism. *Kelletia kelletii* is a large predatory buccinid gastropod commonly found in subtidal kelp forests, rocky reefs and cobble-sand interfaces at depths ranging from 2 to 70 m [34] from Isla Asunción, Baja California, Mexico [35], to Monterey, CA, USA [36]. Rosenthal [34] reported onset of sexual maturity at c. 60 mm in shell length (defined as maximum shell length from the tip of the spire to the tip of the siphonal canal). Kellet's whelks reproduce annually, with egg-laying restricted to late spring and summer. The females deposit masses of egg capsules on benthic hard substrate in which larvae develop for c. 30–34 days. The hatched larvae are pelagic [34]. Laboratory culturing studies resulted in successful metamorphosis of 33% of larvae ($n = 10$) from weeks 5.5 through 9 in the presence of live rock dominated by *Petaloconchus montereyensis* (prey species of *K. kelletii*), as well as 100% of larvae exposed to high concentrations of KCl in weeks 8 and 9; these pilot results suggest a planktonic duration of at least 5.5–9.0 weeks, though the competency window after 9 weeks remains untested (M. Romero and D. Zacherl, unpublished data).

2.2. Laboratory: DVM and Effects of Ontogeny. To determine whether *K. kelletii* larvae undergo nocturnal DVM and if behavior is affected by ontogeny, we cultured replicate batches ($n = 5$) of newly-hatched larvae at 15°C during August and September, 2005. Egg masses laid by *K. kelletii* were hand-collected from McAbee beach, Monterey Bay, California (N36°37.09′ W121°53.82′), via SCUBA at depths of 15–21 meters in August 2005, and transported in coolers to CSU Fullerton. To control for genetic differences only larvae hatching from a single egg cluster were used for this experiment. Seawater (33.2 ppt) used in all experiments was collected from Scripps Institution of Oceanography (La Jolla, California), filtered to 0.2 μm (FSW = filtered sea water), and transported to CSU Fullerton. Egg masses were placed in 4 L clear glass culture jars with lids containing 3 L FSW at 15°C in a temperature-controlled growth chamber illuminated by 6 GE 35 watt high output cool white fluorescent linear lamps (F24T12-CW) with a 12 : 12 light : dark cycle. Every other day the egg masses were removed, culture jars were washed by vigorously rinsing them three times with deionized water and twice with ultrapure water (resistivity > 18.0 MΩ), and the egg masses were returned to jars with fresh FSW. Egg masses were maintained in this way until larvae hatched.

Within 15 hours of hatching, 100 sibling larvae each were placed in replicate cultures ($n = 5$) in 1000 ml glass jars with 800 ml of FSW for a final water column size of ~8 cm diameter (d) × 15 cm height (h). Larvae were reared in the same growth chambers with conditions as described above. Dead or fouled larvae were removed from cultures daily, and the remaining larvae were transferred daily to clean jars containing fresh FSW. After every water change, the total number of larvae in each replicate jar was recorded. We fed larvae a phytoplankton mixture of *Isochrysis galbana* (9,000 cells mL^{-1}) and *Pavlova lutheri* (9,000 cells mL^{-1}) every other day following water changes.

DVM behavior was quantified one day after hatching (i.e., week 1) and once a week through week 5, which approaches the minimum time to competency (Romero and Zacherl, unpublished data). The first observation was made at least 12 hours after water changes and feeding, and occurred four times over a 24 hr period at 0600, 1200, 1800, and 2400 hrs. Observations at 0600 and 1800 hrs occurred an hour after the light source turned on and off, respectively. Initial observations were carried out 20 hours after replicate jars were established. In order to simplify our data into one dichotomous measure, we recorded the number of demersal larvae within each jar. Demersal larvae were defined as any larvae present within 2.5 cm of the bottom of each jar. Unlike subsequent experiments (see below), detailed observations were not recorded on the vertical positions of the remaining larvae, though qualitative observations were recorded.

A two-way ANOVA was used to evaluate the factors time-of-day, ontogeny and time-of-day X ontogeny interactions on the percent of demersal larvae, with replicate as a blocking factor to take into account the repeated measures design. To more intuitively depict DVM behavior graphically (in Figure 1) we converted percent demersal values to percent nondemersal values so that higher values correspond to a higher percentage of larvae in the water columns.

2.3. Laboratory: Effects of Light and Culture Column Height on DVM. To test whether light and culture column height influenced DVM behavior, we cultured replicate batches ($n = 4$) at 15°C during June 2007. Larvae originated from multiple egg masses that were collected at Palos Verdes, CA, USA (N33°42.67′ W118°14.66′), were allowed to hatch in the laboratory at CSU Fullerton, and were cultured together under conditions described above until they were 7-8 days old (hereafter referred to as "week 2 larvae"), except that the light:dark cycle was 16 : 8 to correspond to field conditions during that time of year. One hundred week 2 larvae each were placed in replicate acrylic culture columns ($n = 4$) under two different photoperiod treatments (ambient and dark) and two different water column heights, 9.5 d × 125 h cm (tall) and 9.5 d × 15 h cm (short), filled with 10.1 L and 1.22 L FSW, respectively.

Culture columns were placed in temperature-controlled walk-in incubators (15-16°C). One incubator imitated a natural photoperiod (ambient treatment) with 16 hours of light, from 0500 to 2100 h, followed by 8 hours of dark (16 : 8), while the second experienced 24 h of dark (dark treatment, 0 : 24). Four tall and 4 short columns were randomly placed in each temperature-controlled incubator and were supported by a custom yoke system (resembling a large wooden test tube rack) that enabled us to view the columns from all angles including the bottom. Columns were visually divided into 5 cm increments, with the top and bottom 5 cm sections further divided into 2.5 cm increments. Helical 26 W fluorescent bulbs were placed approximately 7.6 cm away from the top of each ambient light treatment column. Columns were isolated from one another and from the influence of adjacent lights by cardboard dividers and blackout cloth that completely surrounded each column. We placed larvae in columns at 2000 h and larval vertical positions were recorded every 4 h in a 24-hour period starting at 0700 h the next morning. During counting, larvae in both treatments were illuminated with red LED headlamps. *Kelletia kelletii* larvae do not respond to red light (Walker, Zacherl and Hoese unpublished data), as with many other larval invertebrates (e.g., [37]).

For each photoperiod X column height treatment, we calculated the depth center of mass (ZCM) as in Tremblay and Sinclair [38]. ZCM = $\Sigma p_i z_i$ where p_i = proportion of larvae and z_i = distance from top for each depth interval. We then tested the effects of column height, photoperiod and time on ZCM using a three-way full-factorial ANOVA treating replicates as a blocking factor to account for our repeated measures design.

Finally, many factors differed between experiments carried out in 2005 versus 2007, including photoperiod, collection locations for egg masses, time of year, observation intervals, presence of phytoplankton in culture vessels (present in 2005 and absent in 2007), and size, composition of culture vessels (glass versus acrylic), and segmentation of culture vessels into increments. In order to qualitatively compare the results of the two sets of experiments we calculated % demersal larvae in our 9.5 d × 15 h cm (short) culture columns, as in the summer 2005 experiment (see above). Again, to more clearly depict DVM behavior graphically, percent demersal values were converted to percent nondemersal. Larvae of the same age (week 2) were compared to one another.

2.4. Field: Larvae in Surface Plankton Tows. To test whether *K. kelletii* larvae in the field exhibit a distribution consistent with nocturnal DVM behavior, with higher concentrations of larvae at the surface at night compared to day, surface plankton tows ($n = 5$) were conducted at 2400 h and 1200 h. Horizontal tows were conducted perpendicular to the shore using a 0.5-meter diameter plankton net with 333 μm mesh size near the coast of Palos Verdes, CA, USA on July 10, 2007. All tows started between the following two coordinates: N33°43.462′ W118°21.173′ and N33°43.087′ W118°20.106′. The net was maintained at the surface to 1 m below the surface with floats attached to the metal ring of the plankton net while vessel speed was maintained at an average of 2 knots. After eight minutes the net was pulled out of the water vertically and the sample sprayed down with seawater into a cod-end bucket. Using a General Oceanics Inc. mechanical flow meter (Model 2030), we calculated the volume of water sampled per tow as 88.10 ± 1.65 m^3 (SE). Tows were conducted on a clear day and overcast night with surface water temperature averaging 17.2°C. All samples collected were chilled and taken to California State University Fullerton for sorting.

Samples were sorted using dissecting microscopes and all *K. kelletii* larvae were isolated by visual inspection and counted. In order to positively identify *K. kelletii* larvae we compared our plankton tow samples to reference samples of *K. kelletii* larvae collected from Santa Barbara, CA, and cultured in the laboratory through settlement. The count of *K. kelletii* from each tow was converted into larvae/m^3 seawater. Data were log transformed ($\log X + 1$) due to

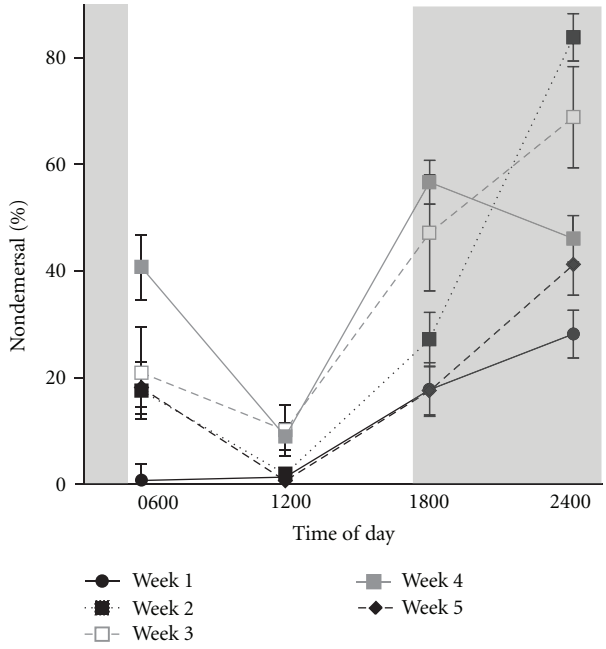

Figure 1: Percentage of *Kelletia kelletii* larvae that were nondemersal throughout a 24-hour period as a function of ontogeny (weeks 1–5) in 8 d × 15 h cm culturing columns ($n = 5$). Grey shading indicates lights off. Error bars represent ± 1 SE.

heteroscedasticity and a Student's t-test was used to compare concentrations of larvae at 1200 h versus 2400 h.

2.5. Light Measurements in the Laboratory and Field. In order to ensure light intensities in laboratory experiments were comparable to light intensities experienced by larvae in the field, we completed profiles of photosynthetically active radiation (PAR, μmol s^{-1} m^{-2}) as a function of depth in laboratory columns and in the field (at 1200 h in partial sun and 1300 h in full sun) off the coast of Palos Verdes, CA (13 July 2007, 33°43′291″N, 118°20′703″W) with an LI-192 underwater quantum sensor and a LI-COR data logger. In the field, PAR was measured in replicate samples ($n = 2$) every 1 m from the surface to 5 m depth, and then every 5 m to 20 m depth. In the laboratory, PAR was measured in the tall columns at multiple depths (1, 25, 50, 75, 100 cm) to ensure that a gradient of light intensity was achieved with depth. In the short columns, we were only able to take measurements at 1 cm below the surface due to size constraints imposed by the sensor.

3. Results

3.1. DVM and Effects of Ontogeny. *Kelletia kelletii* larvae exhibited a daily migration pattern consistent with nocturnal diel vertical migration throughout the five-week period examined (Figure 1). Larvae were found up in the water column at midnight (almost always at the surface based upon qualitative observations made during the experimental period), and demersal during the day, with greater than 80% of larvae demersal at noon throughout their ontogeny. During the observation periods 1 hour after lights were turned on (0600 hr) and off (1800 hr), intermediate proportions of larvae were nondemersal, with larvae scattered throughout the water column and at the surface. There were a higher proportion of demersal larvae during weeks 1 and 5 relative to other weeks, with greater than 60% of larvae being demersal at week 5 regardless of time of day. Last, during week 4, the highest percentage of nondemersal larvae shifted from midnight (2400 hr) to 1800 hr whereas the highest percentage of nondemersal larvae in other weeks peaked at midnight (time-of-day by ontogeny interaction, 2-way ANOVA, $P < 0.0001$, Table 1).

Table 1: Two-way ANOVA testing for effect of time-of-day and ontogeny on *Kelletia kelletii* demersal behavior. DF: degrees of freedom, SS: sum of squares, replicate was treated as random. Bold results indicate significance.

Source	DF	SS	F-ratio	Prob > F
Time-of-day (TOD)	3	32400.91	62.15	**<0.0001**
Ontogeny	4	10562.96	15.20	**<0.0001**
TOD*Ontogeny	12	10183.84	4.88	**<0.0001**
Replicate	1	317.52	1.83	0.18
Error	79	13728.48		
Total	99	67193.71		

3.2. Effects of Light and Culture Column Height on DVM. In both tall and short culture columns, *K. kelletii* larvae exhibited a daily migration pattern consistent with nocturnal diel vertical migration (Figure 2) in both ambient and dark photoperiod treatments, with average larval vertical positioning higher in the water column (i.e., nearer the surface) during night-time hours and lower in the water column during day-time hours (e.g., compare 0300 to 1500 hours in Figure 2, both panels). Larvae in all treatments began their upward migration before 2100 h, while still exposed to light, and migrated downward between 0300 and 0700 h. Larvae in ambient photoperiod treatments in both tall and short columns, had significantly lower average depth center of mass (ZCM) than those in the dark, but only when the larvae were exposed to light (three-way ANOVA, light by time-of-day interaction, $P = 0.01$, Table 2, Figure 2). Post hoc Tukey comparisons ($P < 0.05$) revealed significant differences in ZCM between ambient and dark photoperiods at 0700, 1100 and 1500 h. Column height had no significant effect on ZCM (Table 2).

3.3. DVM in the Field. There were significantly more *K. kelletii* at the surface of the water column at 2400 h, with 0.32 ± 0.08 per m^3, compared to 1200 h, with 0.03 ± 0.02 per m^3 (t-test, $P = 0.018$, Figure 3).

3.4. Light Measurements. In the field, light measurements ranged from 40–1950 PAR at depths from 20 to 0.01 meters below the surface (Table 3). Light intensity diminished with depth; the decline was best described by a logarithmic function $y = -244.1 \ln(x) + 848.68$, with $R^2 = 0.99$. Light intensity in the laboratory columns ranged from 60–298 PAR; the decline in light intensity was best described by the

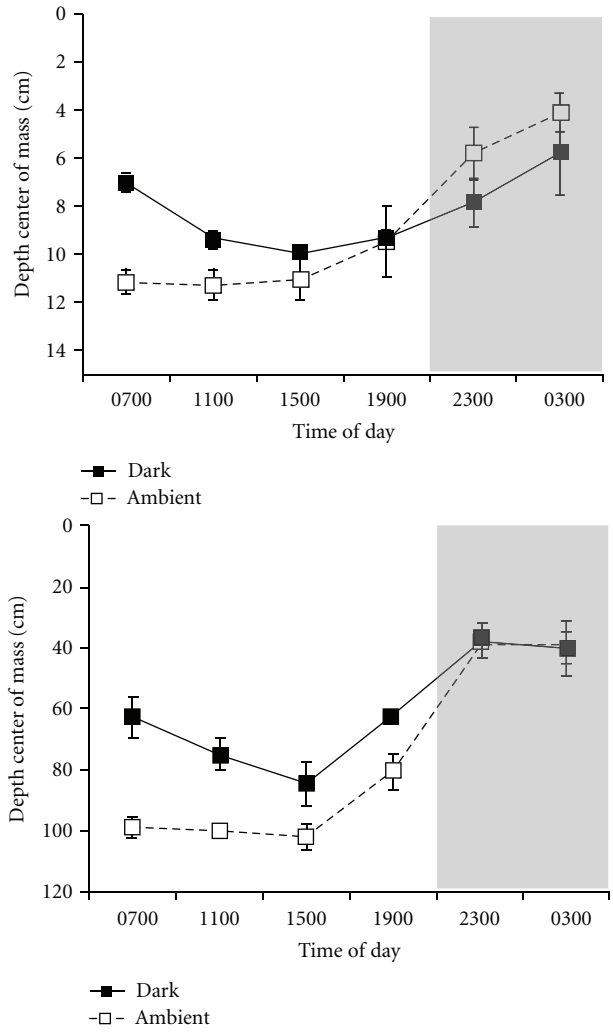

Figure 2: Depth center of mass for *Kelletia kelletii* larvae throughout a 24 hr period in 9.5 d × 15 h cm columns ("short," top panel) and 9.5 d × 125 h cm columns ("tall," bottom panel) in treatments ($n = 4$) of ambient photoperiod (16 : 8 h, open squares) or dark only (0 : 24 h, black squares). Grey shading indicates lights off in the ambient photoperiod. Error bars represent ± 1 SE.

Table 2: Three-way ANOVA testing for effect of light, time-of-day and column height on *Kelletia kelletii* diel vertical migratory behavior. DF: degrees of freedom, SS: sum of squares, Rep: replicate. Rep was treated as random. Bold results indicate significance.

Source	DF	SS	F-ratio	Prob > F
Light	1	0.36	1.80	0.20
Height	1	0.04	0.20	0.66
Light*Height	1	0.24	1.21	0.29
Rep (Light, Height)	12	2.39	3.28	**0.001**
Time-of-day (TOD)	5	11.09	36.59	**<0.0001**
Light*TOD	5	0.99	3.28	**0.01**
TOD*Height	5	0.57	1.89	0.11
Light*TOD*Height	5	0.11	0.37	0.87
Error	60	3.64		
Total	95	19.43		

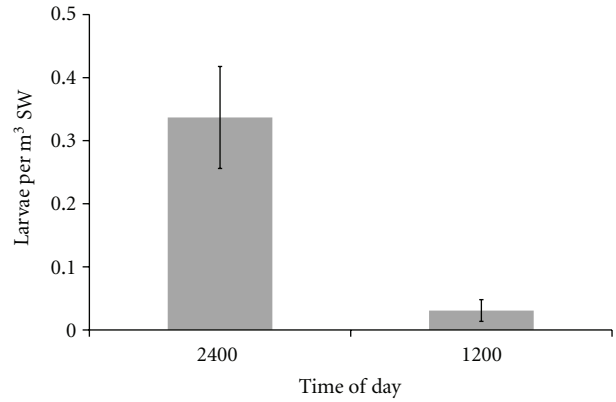

Figure 3: Number of *Kelletia kelletii* larvae per m^3 from replicate ($n = 5$) surface plankton tows conducted at 2400 and 1200 h off Palos Verdes, CA, in June 2007. Error bars represent ± 1 SE.

exponential function, $y = 302.3e^{-1.548x}$ with $R^2 = 0.98$. At the surface of the laboratory columns in the 2007 trials, light intensity was approximately equivalent to that measured in the field at the 10 m depth. For the 2005 trials, PAR at the surface of laboratory columns was equivalent to PAR in the field at 15–20 m depths.

4. Discussion

Both laboratory and field-generated data were consistent with the hypothesis that *Kelletia kelletii* larvae exhibited a classic nocturnal diel vertical migration distribution, with larvae migrating to the surface at night and downward during the day. This pattern has been observed in other veligers, including queen conch, *Strombus gigas* [14] and scallops, *Placopecten magellanicus* [38]. Understanding the functional significance of *K. kelletii's* vertical migration is beyond the scope of this study, though one probable scenario is that the larvae migrate to the surface waters at night to feed on phytoplankton, and return to depth during daylight hours to avoid visual predators or to avoid exposure to high levels of UV radiation [39].

Kelletia kelletii larvae became more demersal as they approached competency—by the time they were 5 weeks old, greater than 60% of all of the larvae were demersal, regardless of time of day. It is unlikely that this ontogenetic shift in vertical distribution is due to a decrease in photopositivity with age, as has been demonstrated in queen conch [14] and invertebrate larvae in general [40], since *K. kelletii* larvae did not exhibit a pattern consistent with photopositivity early in development. Indeed, their vertical positioning during week 1 was most similar to that observed during week 5, with nearly 100% of larvae located in the lowest 2.5 cm of the water column during daytime exposure to light (Figure 1). Their behavior is, however, consistent with a general trend among marine larvae that are approaching competence and preparing to settle into adult habitat—they

Table 3: Photosynthetically active radiation (PAR) measurements in the field during partial-sun to full-sun conditions and in laboratory culture columns. Treatments refer to tall (9.5 d × 125 h cm) and short (9.5 d × 15 h cm) column dimensions. Columns in the 2005 lab treatment measured 8 d × 15 h cm. PAR units are μmol s^{-1}m^{-2}.

Location	Year/treatment	Depth (m)	Range PAR	Avg. PAR ± 1SE (n)
Field	2007	0.01	1898–1950	1924 ± 26(2)
Field	2007	1	860–1050	955 ± 95(2)
Field	2007	5	390–650	520 ± 130 (2)
Field	2007	10	175–335	255 ± 80(2)
Field	2007	15	75–198	137 ± 62(2)
Field	2007	20	40–116	78 ± 38(2)
Lab	2007/tall	0.01	287–292	289 ± 2(3)
Lab	2007/tall	0.25	231–234	233 ± 1(3)
Lab	2007/tall	0.50	150–154	152 ± 1(3)
Lab	2007/tall	0.75	88–93	90 ± 2(3)
Lab	2007/tall	1	60–64	62 ± 1(3)
Lab	2007/short	0.01	242–298	270 ± 28(2)
Lab	2005	0.01	51–144	87 ± 12(10)

are thought to use the additional time near-bottom to sample suitable habitat for settlement [40, 41].

Light, however, had an effect on vertical distributions of our larvae. Throughout ontogeny larvae were demersal when the lights were on and nondemersal with the lights off. In addition, over our 24 hour tracking, average larval vertical position shifted upwards when lights were off. The exact mechanism of how light influenced larval movement is more difficult to characterize. Downward larval movement in response to light could be the result of negative phototaxis, positive geotaxis in response to light cues, or simply negative photokinesis because larvae are negatively buoyant. A horizontal trough with light illumination from the side could be used to isolate the factors from one another [41, 42].

Some observed behaviors in our experiments also suggested the presence of an endogenous rhythm. Larvae in our ambient treatment started migrating upward before the lights went out (Figure 2). Further, larvae in the dark treatment migrated upward during night-time hours and downward during day-time hours, even though no light cues were present. This downward larval migration in the dark treatment was less extreme during day-time hours than in the ambient treatment (Figure 2), suggesting that the presence of light in the ambient treatment induced a behavioral response. An endogenous sunset ascent is part of a theoretical model of DVM behavior in the calanoid copepod, *Calanopia americana* [43], and this ascent may be part of daily activity patterns or may be driven by hunger [44–47]. Downward migration in our study may be cued by a combination of endogenous inactivity, negative phototaxis to high light levels, or both [43]. Because we did not have a sunrise or sunset condition where light level changed gradually, *K. kelletii* larvae in this experiment could not have used relative rates of irradiance change to cue downward migration [12].

The findings of nocturnal DVM behavior in *K. kelletii* were consistent across years (Figure 4) despite different experimental conditions in the laboratory, and these findings were corroborated by our field study of larval positioning.

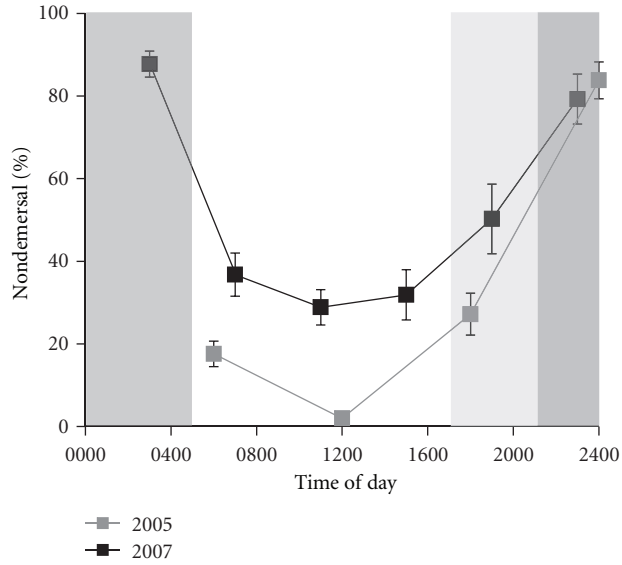

Figure 4: Comparison of vertical migration behavior in 2005 trials versus 2007 trials using larvae of similar ages showing the percentage of nondemersal larvae as a function of time of day. Data collected in 2007 were reanalyzed by converting to % nondemersal in order to make the datasets comparable. Shading indicates timing of light cycles. Light grey shading indicates darkness in 2005. Dark grey shading indicates darkness for both years. Error bars represent ± 1 SE.

In the laboratory, larvae were consistently found higher up in the water column during night-time hours and lower in the water column during day-time hours even with significant differences in the experimental setups in summer 2005 versus 2007 (see Table 4 for a summary of differences). Much has been discussed about experimental artifacts associated with behavior studies in the laboratory (e.g., [18]). Our culture columns provided a gradient of

Table 4: Comparison of experimental and control variables in day-night laboratory trials in jars in 2005 versus short columns in 2007.

Variable	2005	2007
Environment	Incubator	T-controlled walk-in incubator
Light cycle	12: 12	16: 8
Egg collection site	Monterey, CA	Palos Verdes, CA
Egg source	Single female	Multiple females
Data collection interval	6 hrs	4 hrs
Dark phase starting time	1700	2100
PAR range	51–144	242–298
Response factor	% demersal	ZCM
Column dimensions	8 d × 15 h cm	9.5 d × 15 h cm
Phytoplankton present	yes	no

light at realistic PAR intensities relative to field conditions (Table 3). However, light attenuated in our tall laboratory culture columns more quickly than might be expected in the field. Light intensity should attenuate 55–60% in the top 1 meter of the water column; ours attenuated 78–79.5%. The explanation for this stronger attenuation than expected is unclear; we did have a cable attached to our light sensor and it was difficult to keep the sensor vertical, which may have led to some error in our measures. Since our light source was not spectrally matched with natural sunlight, it is possible that some wavelengths that attenuate more quickly than others (e.g., red) may have made up a larger proportion of total light from our light source at the surface. In addition, the wavelengths of light experienced by our laboratory larvae at a particular intensity were not matched spectrally with field conditions. For example at PAR = 60 in the field (at a depth of approximately 20 m), most wavelengths would be blue, compared to the full spectrum of wavelengths potentially experienced by larvae at 60 PAR in the laboratory. Our angular light distribution was also unnatural; while we provided a directional light source, we did not measure whether reflected light in the culture columns affected larval behavior. We also did not mimic other field conditions known to alter larval behavior, such as presence of a thermocline [13], currents [48], and other factors (reviewed in [49] and references therein), which could each potentially override the effects of light and endogenous cues. Future studies should aim to tease out the relative importance of these additional factors on larval behavior. Despite these potential artifacts, our field plankton tows did corroborate the overall pattern of nocturnal DVM witnessed in the laboratory.

Our field measures of larval positioning at the surface at night are consistent with our conclusions that larvae do vertically migrate and that light acts, at least partially, as a cue for larval positioning. However, we do not have evidence that the larvae in the field are demersal during the daytime, as our laboratory results suggest. Based upon our field plankton tows, we only know that *K. kelletii* larvae are not at the surface during the day; we do not know at what depth they are found. Certainly their lower vertical positioning in the laboratory in the presence of light is evident relative to dark treatments (Figure 2, bottom panel). At their average ZCM in the presence of light (approximately 100 cm depth) in the lab, the larvae were exposed to PAR ranging from 60–64, which is equivalent to PAR intensity experienced in the field at approximately 20 m depth on a sunny day. If larval response to light is indeed negatively phototactic or positively geotactic in the presence of light then we predict that Kellet's whelk larvae in the field would descend to a depth of at least 20 meters during midday.

The effective management of any emerging fishery requires some understanding of the connectivity among populations [50] so that important sources of the next generation can be identified [51]. Given the complexity involved with directly tracking larvae of any species from their birth location to their settlement site, many investigators have turned to high-resolution coupled biophysical models to generate a preliminary understanding of larval dispersal trajectories and subsequent connectivity among source populations (e.g., [52]). Knowledge about larval behavior is a critical component of these modeling efforts [3, 53–55]. The data presented here begin to shed insight into *K. kelletii's* larval behavior, suggesting that they undergo extensive daily changes in their vertical positioning possibly on the order of tens of meters. Subsequent studies should emphasize field sampling at multiple depths in the presence of varied flow and thermocline conditions to corroborate the full extent of the daily vertical shifts. Given *K. kelletii's* recently designated status as an emerging fishery, coupled with the knowledge that diel vertical migratory behavior can profoundly affect the dispersal outcomes of larvae, we call for focused attention on this vulnerable fishery.

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