Oecologia (2009) 160:525–536
DOI 10.1007/s00442-009-1327-8

POPULATION ECOLOGY - ORIGINAL PAPER

Darkness as an ecological resource: the role of light in partitioning the nocturnal niche

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Received: 26 September 2006 / Accepted: 9 March 2009 / Published online: 30 March 2009

Abstract Nocturnal behaviors that vary as a function of light intensity, either from the setting sun or the moon, are typically labeled as circadian or circalunar. Both of these terms refer to endogenous time-dependent behaviors. In contrast, the nightly reproductive and feeding behaviors of *Vargula annecohenae*, a bioluminescent ostracod (Arthropoda: Crustacea) fluctuate in response to light intensity, an exogenous factor that is not strictly time-dependent. We measured adult and juvenile activity of *V. annecohenae* throughout lunar cycles in January/February and June 2003. Overnight and nightly measurements of foraging and reproductive behavior of adult *V. annecohenae* indicated that activity was greatest when a critical "dark threshold" was reached and that the dark threshold for adult *V. annecohenae* is met when less than a third of the moon is visible or at the intensity of light 2–3 min before the start of nautical twilight when no moon is illuminated. Juvenile *V. annecohenae* were also nocturnally active but demonstrated little or no response to lunar illumination, remaining active even during brightly moonlit periods. In addition to light level, water velocity also influenced the behaviors of *V. annecohenae*, with fewer juveniles and adults actively foraging on nights when water velocity was high (>25 cm/s). Our data demonstrate that the strongest environmental factor influencing adult feeding and reproductive behaviors of *V. annecohenae* is the availability of time when illumination is below the critical dark threshold. This dependence on darkness for successful growth and reproduction allows us to classify darkness as a resource, in the same way that the term has been applied to time, space and temperature.

Keywords Bioluminescence · Ostracod · Dark threshold · Lunar cycle

Communicated by Pete Peterson.

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Introduction

Darkness is a resource and an environmental condition that is as important as light to almost all living organisms. In photosynthesizing plants or organisms exposed to ultraviolet radiation, periods of darkness provide time for photorepair, allowing over-stimulated cells to recover and mend (Sutherland 1981; Mitchell and Karentz 1993; Friedberg et al. 1995). Without a period of darkness, these organisms experience reduced growth and lower reproductive success (Britt 1996; Grad et al. 2001). Additionally, because natural light and dark cycles have existed throughout evolutionary time, many organisms have evolved behaviors that are cued by or are dependent upon darkness. In both terrestrial and aquatic systems the germination of seeds and hatching of eggs depend on photoperiod cues (Stross and Hill 1965; Aiken 1969; Venable and Lawlor 1980). In marine systems, timing of spawning in corals, other invertebrates and fishes often corresponds to specific lunar light conditions (Taylor 1984; Babcock et al. 1986; Lessios 1991). Some planktonic

invertebrates and larval fishes exhibit diel vertical migration (DVM) and increased feeding activity at night when hunting by large visual predators is inhibited (Fisher and Bellwood 2003; Hays 2003). Bioluminescent organisms exhibit some of the most apparent adaptations to darkness. For these highly evolved organisms, darkness provides the veil under which their complex reproductive, defensive and communicative behaviors take place.

Bioluminescent organisms represent <10% of the fauna in littoral marine communities (Morin 1983), yet their requirement for dark conditions is representative of many of the non-bioluminescent nocturnal organisms that share this habitat. Most bioluminescent organisms use light for defense, to warn predators to stay away or to alarm nearby conspecifics (Morin 1983; Herring 1990; Hastings and Morin 1991). In many of these same species, bioluminescence is also used for mate location during courtship and reproductive behaviors (Morin 1983; Herring 1990; Hastings and Morin 1991; Widder 1999). The level of darkness required for bioluminescence to be effective depends upon a lower limit of solar, lunar, or artificial illumination (Longcore and Rich 2004; Rich and Longcore 2006). In this sense, the level of darkness acts to partition the habitat that bioluminescent organisms are able to use both temporally and spatially. The same is likely true for all organisms that are crepuscular or nocturnal.

Vargula annecohenae Torres and Morin 2007 is a bioluminescent ostracod found in sub-tidal grassbeds of the western Caribbean and strongly depends on dark conditions for successful reproduction. During courtship, adult *V. annecohenae* males forsake their mostly benthic life style and swim up in the water column emitting packets of bioluminescent chemicals in a species-specific display pattern that attracts females (Morin and Cohen 1991; Torres and Morin 2007; Rivers and Morin 2008). These displays are produced and visible only when the ambient light conditions in the grassbeds reach a critical "dark threshold". This threshold is related to the level of lunar or solar illumination and is well below the light intensity produced by a full moon (Fig. 1a). To understand fully the role that ambient light and lunar cycling play in the life cycle of this highly abundant resident of marine grassbeds, we monitored foraging and courtship behaviors during two lunar cycles in the grassbeds surrounding South Water Caye, Belize. We proposed three alternative hypotheses to explain the potential relationship between *V. annecohenae* behavior and the lunar cycle.

1. The "dark threshold hypothesis" proposes that feeding and reproductive activity occur at a constant elevated intensity whenever light levels are below a critical dark threshold (Fig. 1b).
2. The "circalunar burst hypothesis" suggests that female *V. annecohenae* molt and become receptive cyclically due to endogenous, circalunar rhythms (e.g., at full moon) similar to other groups of crustaceans (Reaka 1976; Franke 1986), thus creating peak breeding periods under specific light and tidal conditions (Fig. 1c). Based on the circalunar burst hypothesis, adult reproductive behaviors should be cyclical while juvenile and adult foraging behaviors should be unaffected.
3. The "dark threshold and reproductive potential hypothesis" is based on our knowledge that juvenile females molt into receptive adults and adult females carrying broods of embryos release their offspring on a regular basis (Gerrish and Morin 2008). According to this third hypothesis, during highly illuminated periods such as daytime or the nights just before the full moon, receptive females accumulate because the dark threshold, which is required for male reproductive displays, is never reached. When the dark threshold is finally crossed, we expect the activity of adults to be greatest when the highest numbers of receptive females are available or when the reproductive potential is greatest (Fig. 1d). Juvenile behaviors should demonstrate little to no response.

Materials and methods

Study system

Vargula annecohenae is a small-bodied (~2 mm) bioluminescent ostracod (Crustacea: Ostracoda: Cypridinidae) that is found in high abundances (>500/m^2) in grassbeds surrounding islands on the Belize barrier reef (Torres and Morin 2007). Like most cypridinid ostracods, *V. annecohenae* remains primarily on or in the sea bed; however, adult *V. annecohenae* males enter the water column on most nights to produce their bioluminescent courtship displays meant to attract conspecific females (Morin and Cohen 1991). Similar to that of other cypridinid ostracods, the life cycle of *V. annecohenae* includes five juvenile instars and one terminal adult instar (Cohen 1983; Cohen and Morin 1990; Torres and Morin 2007). All instars can be accurately identified based on the length to width ratio of their carapace (Gerrish and Morin 2008). Sexual dimorphism becomes apparent in stage five and remains obvious through the adult stage. Based on animals collected from the field and maintained in laboratory cultures, females release eggs approximately 8 days following mating. Fertilization takes place as the eggs are extruded into a "brood pouch" located within the marsupium of the female carapace and embryos develop for an average of 18 days before release (Gerrish and Morin 2008). Stage one juvenile

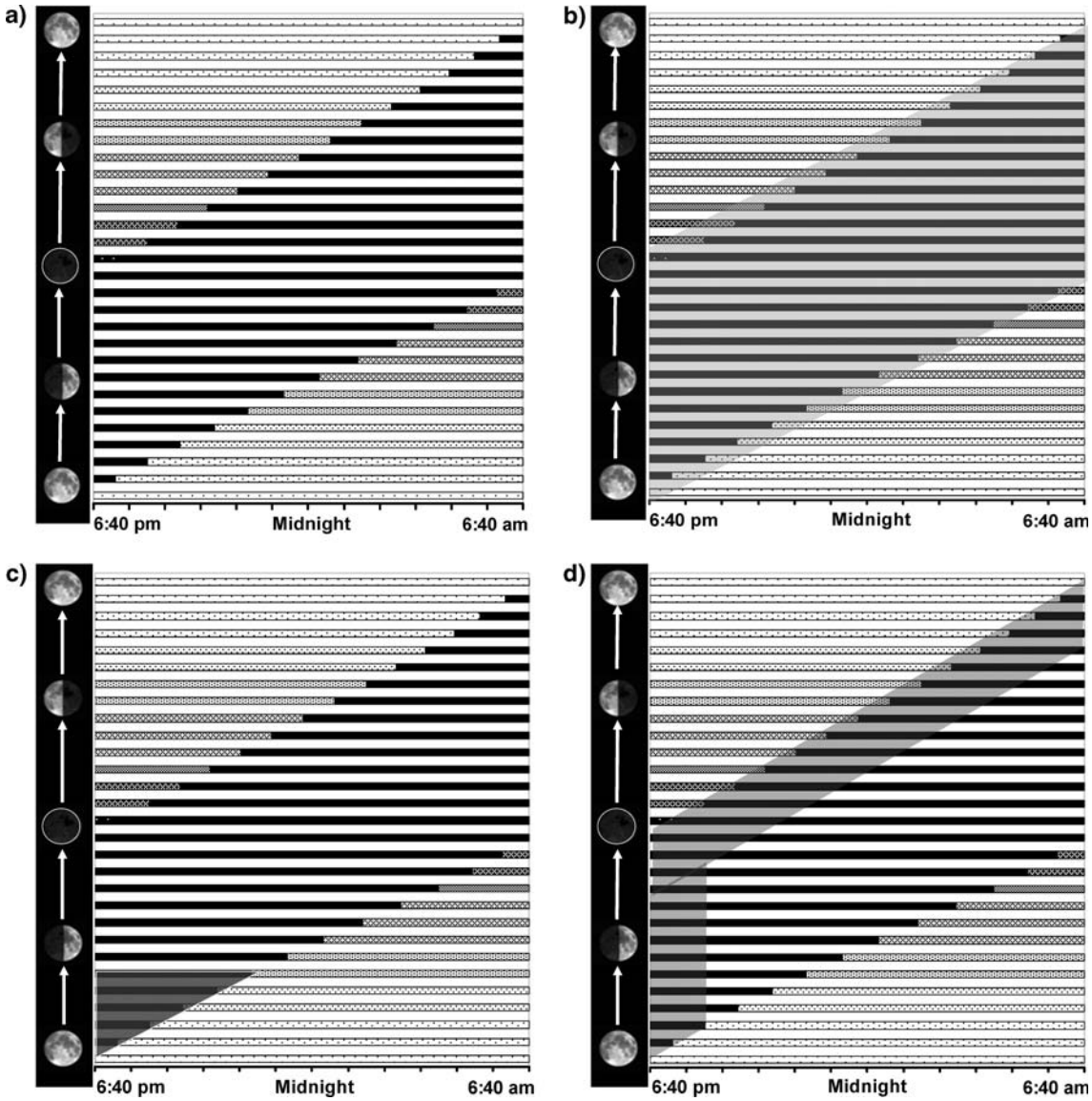

Fig. 1 **a** The intensity of darkness each night during the lunar cycle in relation to moon rise and set. **b** The "dark threshold hypothesis": the *highlighted area* indicates that *Vargula annecohenae* activity is expected to be similar in intensity at any time when light levels are below the "dark threshold". **c** The "circalunar burst hypothesis": here depicted for the few days following the full moon, includes one intense burst of activity and then little to no activity for the rest of the lunar cycle. **d** The "dark threshold and reproductive potential hypothesis": increased adult foraging activity and reproductive activity is expected during times when the highest number of reproductively available females is present in the population. *Dark bars* represent full darkness, *stippling* represents a gradation of darkness and *shaded areas* represent periods of theoretical activity for *V. annecohenae*

instars are released from the adult females as benthic "crawl-away" juveniles. These characteristics and the fact that *V. annecohenae* can be captured in abundance using baited foraging traps make them a tractable system for studying demographic behaviors.

Nightly and overnight observations

Sampling took place just off the south beach of South Water Caye, Belize (16°48′45″N, 88°04′57.5″W) in ~1.5-m-deep water where turtle grass (*Thallasia testudinum*) dominated the substrate. To measure the relative activity of juvenile and adult *V. annecohenae* across time periods, we used the number of animals captured in baited traps as an estimate of foraging activity. Traps were created out of cylindrical polyvinyl chloride piping (7 cm long × 3.8 cm diameter), containing inverted funnels of 75-μm nylon mesh at both ends. Screw caps lined with plastic window screening held the funnels in place and kept out the majority of large, unwanted carrion foragers while allowing all sizes of ostracods to pass through. A brass clip was attached to each trap using a plastic cable tie and traps were deployed

containing a piece of dead fish (≈1 cm^3) to attract scavenging ostracods. Three traps were set each night from 26 January to 21 February 2003 during the hour immediately following nautical twilight. While snorkeling, we clipped traps at 2-m intervals along a submerged line. All individuals captured in traps were counted and aged based on documented age-size relationships for each instar (Gerrish and Morin 2008). The data were then corrected for the time that each trap was deployed in the water (50–65 min) to provide foraging activity estimates (number captured per hour). In January 2003, we also monitored foraging activity overnight on three occasions as the moon fell and rose in the sky. Three traps were deployed every hour throughout each night and all individuals captured were counted and assigned to instar based on size measurements.

We characterized the environment at the time of capture by measuring water velocity and temperature. Velocity measurements were taken by releasing 10 cm^3 of fluorescence dye hydrated with sea water at a flagged location. Dye was released just above the top of the turtle grass and allowed to move through the water. The location of the front edge of the dye was marked with another flag after a recorded duration of time. The distance between flags represents the distance the dye moved over a known duration of time and this provided our water velocity estimate (centimeter per second). Temperature was recorded using a mercury thermometer held at the turtle grass–water column interface (10–15 cm from bottom).

The other environmental variable of interest is represented by percent darkness, and is an estimate of the relative level of lunar illumination throughout the lunar cycle at the time of our sampling. Although directly measuring illumination and quantifying cloud cover at the time of sampling would have been the most accurate means of acquiring light level estimates, we lacked the equipment and had focused on the phase of the moon as a factor rather than lunar illumination itself. Therefore, we estimated percent darkness based on the lunar photometric model of Helfenstein and Veverka (1987). This model integrates Hapke's 1986 equation that is fit to the lunar disk-integrated visual lightcurve and disk-resolved data, with the reflective characteristics of varied moon surface substrates and terrains (Helfenstein and Veverka 1987). Using this lunar photometric model, we calculated whole-disk brightness for each phase angle of the moon with 0° being the "ideal" full moon and 180° being the "ideal" new moon. Next, we assumed the whole-disk brightness of the moon at 2° phase (the smallest phase seen from Earth outside lunar eclipse) as a practical reference brightness for the full moon. The level of brightness at the full moon, 2° phase, was assigned 0% darkness and the brightness level at the new moon, 179° phase, was assigned 100% darkness. Using this relationship, we derived a "phase curve" to estimate the relative level of darkness (% darkness) across phase angles for the entire lunar cycle. Based on almanac data from NASA (http://eclipse.gsfc.nasa.gov/phase/phasecat.html) regarding the timing of full and new moon for our January/February and June sampling periods, we could identify the phase angle and percent darkness of the moon that corresponds with our sampling period each night.

From 3 June to 30 June 2003, in addition to monitoring foraging activity and environmental conditions nightly, we also monitored ostracod display activity. Quantifying the number of displays in an area proved difficult due to the patchy nature of displays; therefore we estimated densities based on catch-per-unit effort. Because we were most interested in comparing display densities between nights, the same researcher sampled displays each night within the same general region (40 m^2) and within a homogeneous habitat of similar grass density and depth. Displays were sampled using a net swept upward through a single bioluminescent display, capturing the displaying male, and we counted the number of these sweeps possible in a 3-min period. Alternatively, when displays were heavy we recorded the amount of time it took to collect 50 displays. Assuming a constant swimming, detection and collection speed by the researcher, our estimate of the number of displays captured over time is comparable from night to night. Three measures of activity were made approximately 20 min apart during the first hour of displays each night to document any increase or decrease in display behavior.

During January 2004, we monitored female receptivity and female reproductive activity in field populations. If females are becoming receptive or reproducing cyclically due to intrinsic circalunar cues, we would expect that all females, regardless of the timing of collection, would closely overlap in timing of impregnation, brooding and birthing. To test if females become receptive and reproduce cyclically throughout the lunar cycle, we collected females for 20 consecutive nights and monitored their reproductive characteristics in the laboratory during the following month. Each night, 24 impregnated, pre-brooding females were isolated from foraging traps. To minimize exposure of females to males in the traps and limit forced copulation during confinement, we removed traps from the field and isolated the females as soon as we could determine that 24 females had been collected. The duration of trapping varied from 5 to 20 min depending on the activity level of females each night. Captive females were placed in 12-well tissue culture plates (well = 22.6 mm diameter × 20 mm deep), fed fresh dead fish ad libitum, provided fresh seawater every 2–3 days, and kept under photoperiod (≈12 h light:12 h dark) and temperature (≈26°C) conditions similar to those found in their natural habitat. Knowing that females hold eggs internally for an average of 8 days post-mating (Gerrish and Morin 2008), we monitored females

for 15 days following collection to quantify the proportion of females that brooded within that period. Assuming that all successfully impregnated females brood eggs, the proportion of females brooding each night is presented as it relates to the percent of impregnated females at the time of capture.

Data analysis

Foraging activity and water velocity were natural log transformed for analysis**.** Using the general linear model procedure (proc. GLM) in SYSTAT 9.0 (SPSS), we tested how the foraging activity of each instar of *V. annecohenae* varied between nights throughout the lunar cycle. Instar (including male and female classes for adults), date of sampling, and age class (juveniles vs. adults) were treated as fixed variables and the number of animals captured in foraging traps was our response variable. Based on non-significant interaction terms, instars that varied similarly across dates were grouped for further analysis. Next, in proc. GLM (SPSS Science 1999), we used a three-way ANOVA to test the interactions among sampling period (January/February and June), ostracod stage (adults vs. juveniles) and darkness (full vs. partial). Each factor was treated as fixed and categorical. Any interactions that were significant with a probability of 0.05 were further analyzed using post hoc Scheffe-corrected pairwise comparisons. Foraging data were then regressed using a mixed model in proc. GLM in SYSTAT 9.0 (SPSS) against the continuous variables percent darkness and water velocity, and with season maintained as a fixed variable in the model. Based on significant interactions observed in the fully fixed model, the mixed regression was applied separately for juveniles, adults on 100% dark nights and adults on nights when darkness varied. Water velocity and darkness were not correlated ($\text{Pearson}_{n=40} = 0.097$) and were therefore treated as independent predictor variables. Temperature did not vary within January/February or June and is therefore not used as a predictor of ostracod density, but a paired *t*-test was used to test significant differences in temperature between January/February and June. Models began as fully explicit, testing all factors and interactions. We removed interactions and then main effects in a stepwise fashion (criteria $P = 0.150$) to isolate the models that explained the most variation. Because only one measure of darkness and velocity was taken on each night, we used average ostracod density collected in the three foraging traps as the response variable in the GLM.

Results

Very few individuals of the youngest (first or A-V) *V. annecohenae* instar were captured; therefore, the youngest instar was omitted from the analysis. We also observed significant differences in the number of instars A-IV, A-III, A-II and A-I that were captured ($P < 0.001$; Table 1). But the foraging activity of each of the four oldest juvenile instars of *V. annecohenae,* when grouped within the juvenile age class, varied in a similar direction and intensity across the dates sampled ($P = 1{,}000$; Table 1) throughout both the January/February and June sampling periods. Based on the non-significant interaction between day sampled and instar within age class we treated all juveniles cumulatively for further analysis. Adult male and female instars were also grouped because they varied similarly in density across dates sampled ($P = 1.000$; Table 1). Juveniles and adults varied significantly in how their foraging densities changed across the dates sampled and were therefore analyzed separately ($P < 0.001$; Table 1). Juveniles varied in a similar fashion across the lunar cycle, whereas adults were captured less frequently in foraging traps on the nights approaching the full moon, when the sky is brightly lit, during our sampling period (the first hour after nautical twilight each night) (Fig. 2). Additionally, because we captured some of the highest densities of *V. annecohenae* in both January/February and June on dark nights that followed many nights of previous sampling, and the grassbed habitat is quite uniform and extends many tens of meters in all directions, we assume our repeated sampling within a region does not influence our inferences regarding the patterns observed in foraging activity (Fig. 2).

On half of the nights sampled, no moon was present in the sky at the time of collection (Fig. 1a; 6:30 to 7:30). There was a significant interaction between age class (juvenile or adult) and darkness (100% or <100%) ($P = 0.005$; Table 2). In post-hoc hypotheses tests, juveniles had no significant difference in mean activity between nights when no moon was present (=100% darkness) and moonlit nights (<100% darkness) (Fig. 3; Scheffe $P = 0.863$). In contrast, adults were captured in greater densities on nights when no moon was present (Fig. 3; Scheffe $P = 0.020$). Because there was a significant difference and because the large

Table 1 ANOVA to test if the number of each instar (A-IV, A-III, A-II, A-I, adult females, and adult males) captured varied significantly among sampling days and whether the number captured differed between instars when grouped within age classes of juveniles and adults

Source	*df*	MSE	*F*-ratio	*P*
Day sampled	51	16,850.818	6.756	0.000
Instar	5	36,291.174	14.550	0.000
Day sampled × age class	51	7,672.171	3.076	0.000
Day sampled × instar within age class	204	1,016.645	0.408	1.000
Error	595	2,494.173		

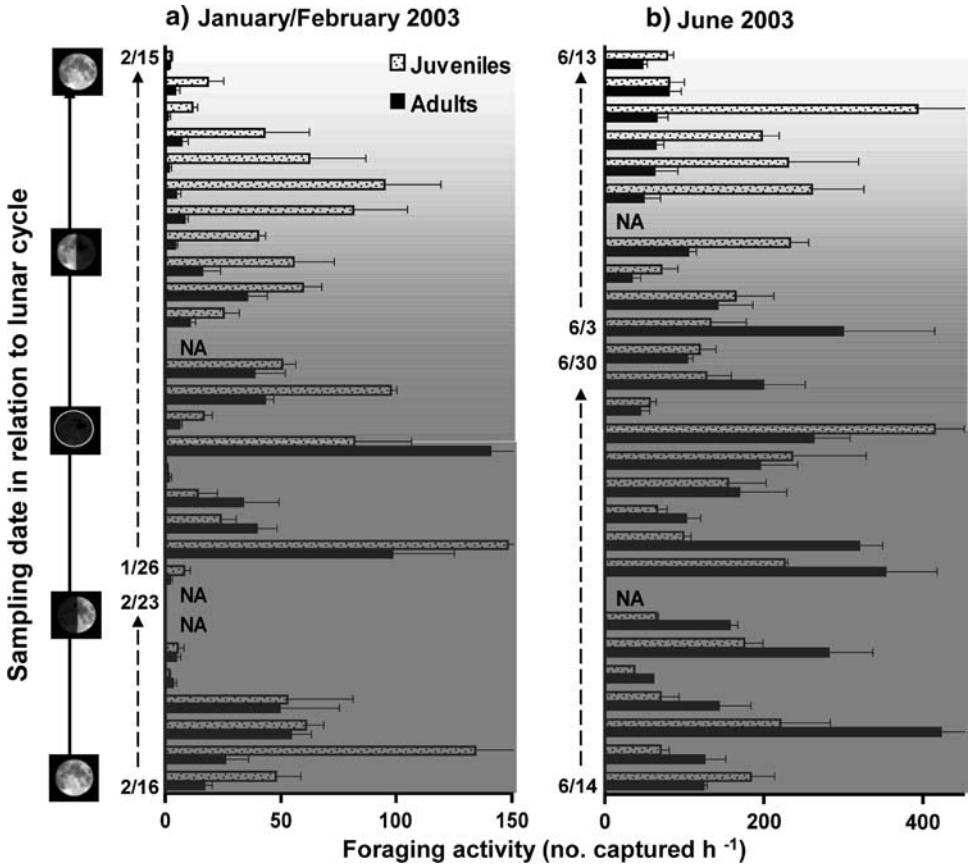

Fig. 2 Foraging activity (mean ± SE) of juvenile (*stippled bars*) and adult (*dark bars*) *V. annecohenae* during **a** January/February and **b** June 2003. Sampling day in relation to the phase of the moon is represented on the *y*-axis with the *lowest bar* representing the first day after the full moon, **a** 16 February and **b** 14 June. Dates of collection are also labeled for the beginning and end of each sampling cycle. *Shading* represents the relative percent darkness available to the ostracods during foraging activity measurements with *darkest areas* equal to 100% dark. *NA* Not available (nights when sampling did not take place)

number of measurements at 100% darkness would heavily weight the analyses, adult data were partitioned into two groups, nights with <100% darkness and nights when darkness was equal to 100% (Fig. 4).

Temperature varied minimally within January/February (25.5–27.0°C) and June (30.0–31.1°C) but did differ significantly between months ($t = 12.894$, $df = 26$, $P < 0.001$) and may be one physical factor contributing to variation in *V. annecohenae* feeding and reproductive activity attributable to sampling period (January/February vs. June). Water velocity was lower in June (4–21 cm/s) and varied much less than in January/February (5–75 cm/s). Because velocity varied both within and between sampling periods it was included as a variable in the analysis of both adult and juvenile feeding activities.

Table 2 ANOVA of sampling period (January/February and June), darkness (=100% or <100%), and age class (juvenile or adult)

Source	*df*	MSE	*F*-ratio	*P*
Sampling period (SP)	1	364,646.657	67.480	0.000
Age class	1	6,417.961	1.188	0.279
Darkness	1	14,812.626	2.741	0.101
SP × age class	1	762.183	0.141	0.708
SP × darkness	1	3,716.750	0.688	0.409
Age × darkness	1	44,602.585	8.254	0.005
Darkness × SP × age class	1	19,915.992	3.686	0.058
Error	96	5,403.768		

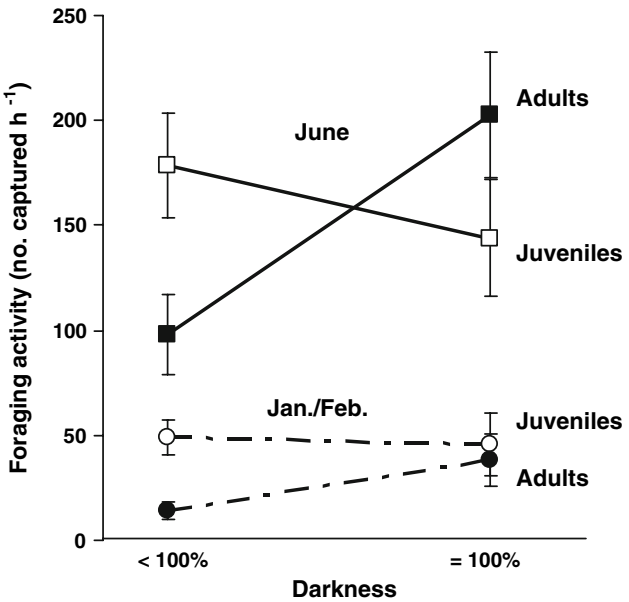

Fig. 3 Average adult (*filled square*, *filled circle*) and juvenile (*open square*, *open circle*) *V. annecohenae* foraging activities during January/February (*dashed line*) and June (*line*) on nights when a moon was present in the sky (darkness < 100%) or on nights when no moon was present in the sky (darkness = 100%)

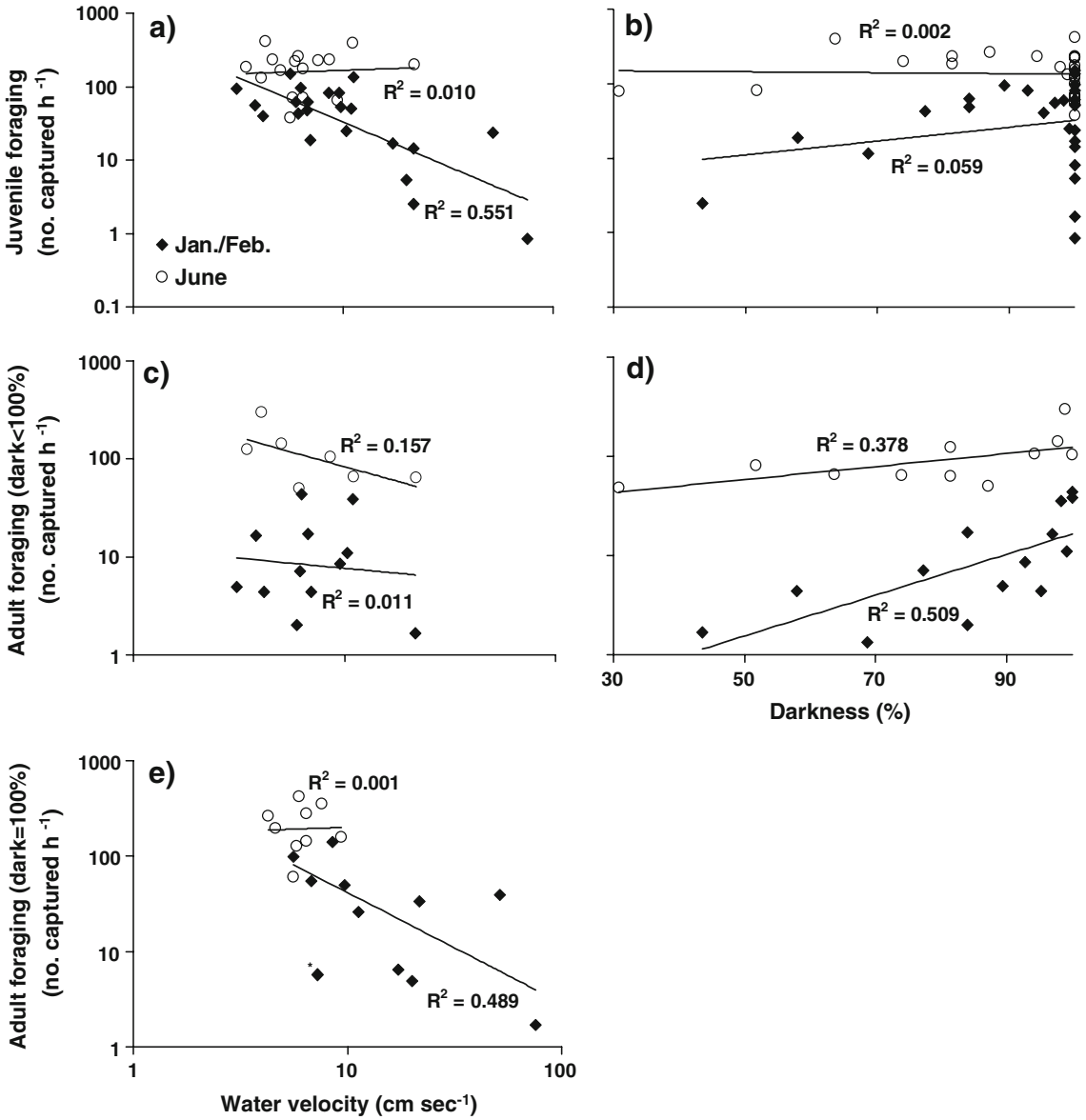

Fig. 4 *V. annecohenae* foraging activity in January/February (*open circle*) and June (*filled diamond*) in relation to water velocity (**a**, **c**, **e**) or darkness (**b**, **d**). Activity and water velocity varied across exponential scales and therefore both variables were transformed using natural log for linear analysis of **a** juvenile *V. annecohenae*, **c** adult *V. annecohenae* when darkness is <100%, and **e** adult *V. annecohenae* when darkness is equal to 100%. Because darkness did not vary on nights when darkness equaled 100% the relationships between darkness and *V. annecohenae* activity were explored only for **b** juveniles and **d** adult *V. annecohenae* when darkness was <100%

For juvenile activity, there was a marginally significant interaction between water velocity and sampling period (Fig. 4a; Table 3; $P = 0.067$) and a significant interaction between percent darkness and sampling period (Fig. 4b; Table 3; $P = 0.006$). In January/February, there was no relationship between darkness and juvenile ostracod activity (Fig. 4b; $r^2 = 0.0588$, $P = 0.233$). In June, slightly fewer juvenile ostracods were captured on darker nights (Fig. 4b; $r^2 = 0.0016$, $P = 0.848$). Yet, on very bright nights (darkness <10%) very few juveniles were captured (Fig. 4b). No relationship was observed between juvenile activity and water velocity in June (Fig. 4a; $r^2 = 0.010$, $P = 0.812$); however, in January/February, fewer juvenile *V. annecohenae* were captured on nights when velocity was high (Fig. 4a; $r^2 = 0.551$, $P < 0.001$). The best fit model predicted 75.0% of the variation observed in juvenile activity and included sampling period, water velocity, darkness and the interactions of sampling period with water velocity and darkness.

On nights when a moon was present in the sky (darkness < 100%), water velocity, the interaction of velocity and sampling period, and the three-way interaction

Table 3 Reduced[a] general linear models (*GLM*s) for juveniles, adults when darkness is <100%, and adults when darkness equals 100%

Source	*df*	MSE	*F*-ratio	*P*
Juveniles ($r^2 = 0.750$)				
Sampling period (SP)	1	1.359	2.792	0.104
Water velocity	1	4.164	8.553	0.006
% Dark	1	0.003	0.006	0.937
SP × % dark	1	4.177	8.579	0.006
SP × water velocity	1	1.746	3.586	0.067
Error	33	0.487		
Adults when darkness <100% ($r^2 = 0.803$)				
SP	1	8.734	17.483	0.000
% Dark	1	7.434	14.879	0.001
SP × % dark	1	2.595	5.194	0.033
Error	22	0.500		
Adult GLM = 100% darkness ($r^2 = 0.691$)				
Water velocity	1	28.511	38.02	0.000
Error	17	0.750		

[a] Full models contained sampling period, water velocity and % dark (except for adults at 100% dark) and were reduced in a stepwise fashion

between water velocity, sampling period and percent darkness did not explain a significant amount of variation in adult activity; however, the negative trend observed with fewer individuals captured under higher water velocity conditions was maintained (Fig. 4c). In both June and January/February, increased numbers of adult *V. annecohenae* were captured in feeding traps on darker nights (Fig. 4d; Table 3; $P < 0.001$). The strength of this interaction varied significantly between January/February and June (Fig. 4d; Table 3; $P = 0.033$). The best-fit model to predict adult *V. annecohenae* densities on moonlit nights included sampling period, darkness and their interaction ($r^2 = 0.803$). Incorporating quantitative measures of cloud cover would likely have improved the resolution of this model. Qualitative categorizations of clear vs. cloudy or overcast (hazy sky/not true cloud formation) were made at the time of data collection. Very cloudy conditions were documented on 27 January and 17 February 2003, and correspond to dates when high densities of ostracods were captured (Fig. 2a). Overcast conditions occurred from 29 January to 1 February 2003 but do not have a definitive interaction with ostracod densities. In June, cloudy conditions were noted on the 9th, 10th and 26th and may correspond to higher densities on those nights (Fig. 2b).

For adults, on nights when no moon was present in the sky during sampling (darkness = 100%), a greater average of *V. annecohenae* adults was collected overall (Fig. 3); however, there was high variation in densities captured among dark nights (Fig. 2b). Water velocity explained a significant portion of this variation and only its main effect was significant (Fig. 4e; $P < 0.001$, $r^2 = 0.691$). In June there was very little variation in velocity and we observed less variation in adult activity (Fig. 4e; $r^2 = 0.001$, $P = 0.934$); however, in January/February when water velocity varied most, fewer adult *V. annecohenae* were captured when water velocity was high (Fig. 4e; $r^2 = 0.489$, $P = 0.024$).

Display activity

Reproductive display behavior by males follows a threshold response with 0 displays occurring on nights when darkness is <95%. Higher display activity was observed on darker nights with the highest activity observed on 100% dark nights (Fig. 5). Additionally, on 100% dark nights when displays occurred, displays began 1–2 min before the start of nautical twilight, peaked and then dropped off over the following hour (Fig. 6).

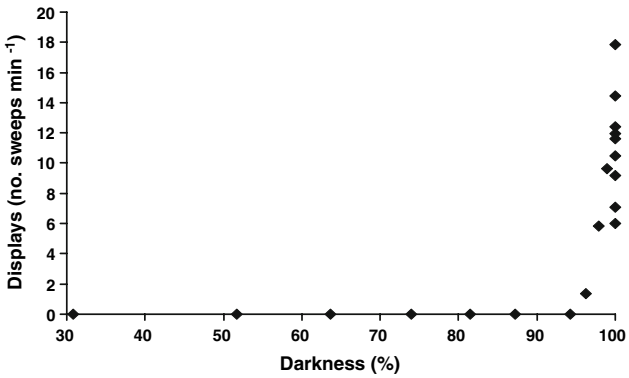

Fig. 5 Reproductive displays throughout the June lunar cycle. Displays did not occur on nights when darkness had not reached the dark threshold, which occurs at approximately 95% darkness

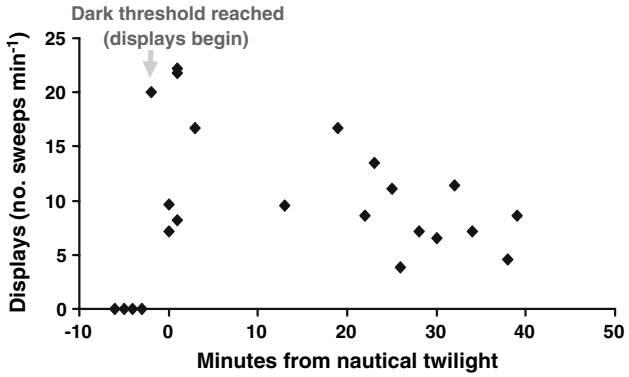

Fig. 6 Data from sweep samples indicate that reproductive displays began within 2–3 min of the start of nautical twilight, quickly peaked in number, then decreased in frequency continuously over the 1-h sampling period. The *gray arrow* represents the approximate timing when the dark threshold occurs relative to display abundance

Overnight activity

Hourly measures of *V. annecohenae* activity, based on data from feeding traps set throughout the night, varied in relation to the timing of moon rise or moonset. Juveniles were active throughout the night on 12 January 2003 and exhibited lowest activity during dark periods when adults peaked in activity. Adults became active as the moon set and were most active during the first hour following moonset, and then the number captured decreased each subsequent hour (Fig. 7a). Both adult and juvenile activity remained low on 19 January 2003 after the nearly full moon rose at 7:00 p.m. (Fig. 7b). Low numbers of both juveniles and adults were actively foraging on 25 January 2003 and no clear patterns are visible in the data, except a small burst of activity just prior to sunrise (Fig. 7c).

Female receptivity

If females were impregnated by forced copulations that occurred in traps while sampling, we would expect most females to brood 7–8 days following their capture. Instead, females brooded at any time between day 1 and day 8 after their collection, and there was no sudden increase on day 7 or 8. Overall, no pattern in female receptivity or impregnation is discernable. On average 60% of females were already gravid at the time of capture and there was no significant change throughout the lunar cycle (Fig. 8; $P = 0.754$).

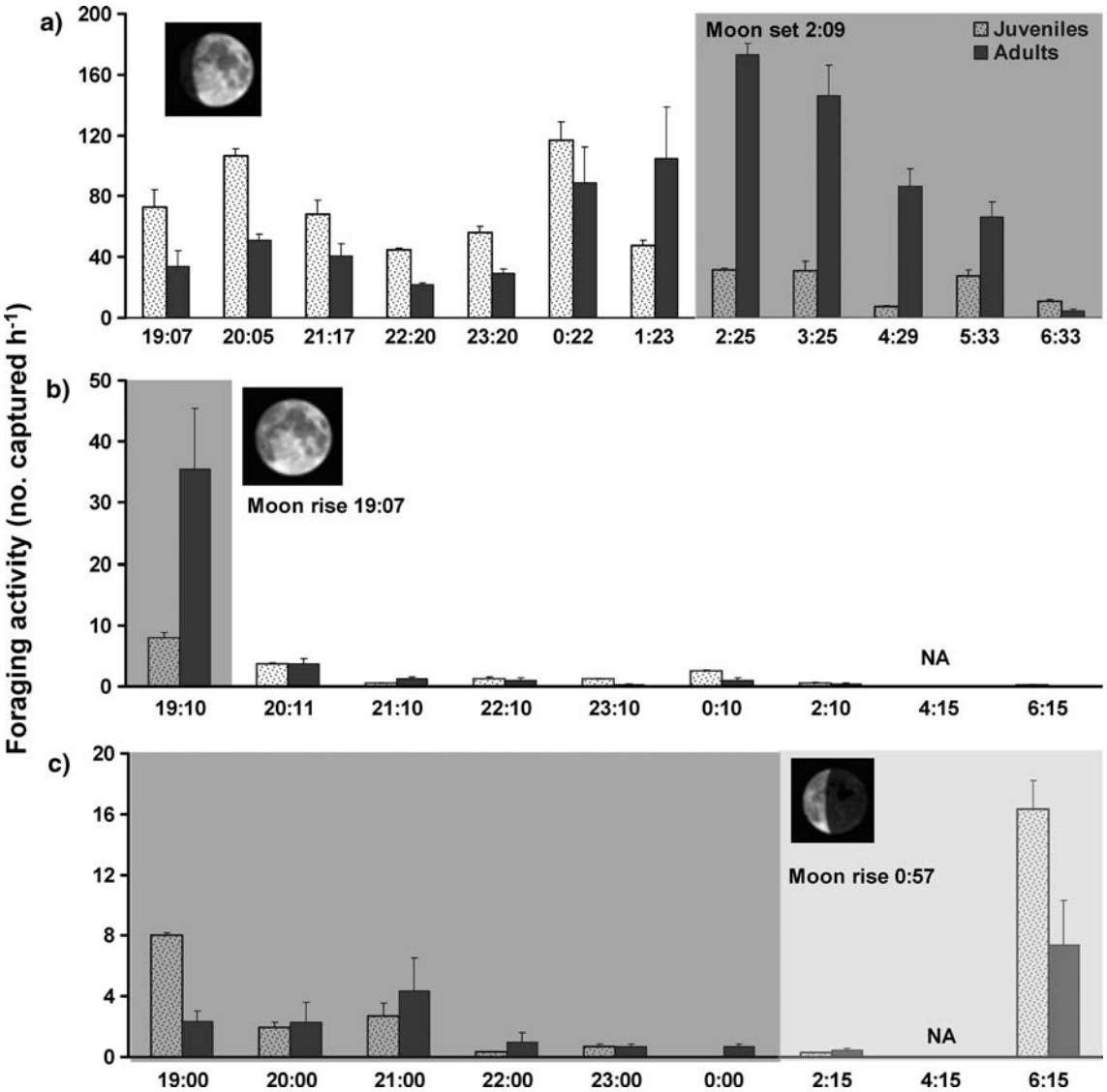

Fig. 7 Overnight foraging activity (mean ± SE) of juvenile (*stippled bars*) and adult (*solid bars*) *V. annecohenae* on **a** 12 January (3/4 moon), **b** 19 January (nearly full moon) and **c** 25 January (1/3 moon) 2003. *Shading* represents darkness at each hour and *insets* show the phase of the moon illuminating the sky during moonlit hours. Time of moon rise or moon set is noted in each panel

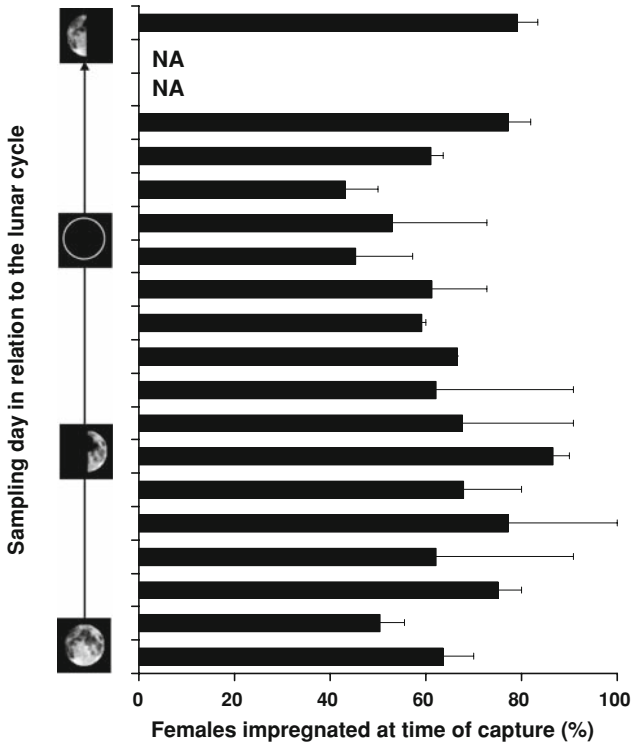

Fig. 8 Frequency of female impregnation during the January 2004 lunar cycle (mean ± SE). Females were used to calculate this percentage only if they survived greater than 20 days in culture (n = 6–12, depending on survival in culture)

Discussion

Feeding and reproductive behaviors in adult *V. annecohenae* begin when the dark threshold is reached, and this threshold is roughly equivalent to a one-third moon being present in the sky or to the intensity of light 2–3 min before the beginning of nautical twilight on nights when no moon is present in the sky. The behavioral responses of *V. annecohenae* to this dark threshold are independent of timing, as indicated during overnight sampling when adults become active in the middle of the night as the moon sets. Additionally, on cloudy nights reproductive displays and foraging behavior appear to increase if a partial moon is well covered and the necessary dark conditions are met (G. A. Gerrish and J. G. Morin, personal observation). Furthermore, adult male *V. annecohenae* can be triggered in the laboratory to begin displaying at any time throughout a day by placing them into completely dark conditions (Rivers and Morin 2008). For *V. annecohenae* the exogenous cues provided by the dark threshold appear to outweigh any endogenous rhythms effecting behavior. This uncoupling of physical cues and chronological rhythms occurs in multiple organisms (Danks 2005), and has even been shown to have underlying genetic controls (Mathias et al. 2006). These observations bring into question the very common labeling of light level as a Zeitgeber. Although in nature, light and dark inherently have temporal or circadian associations, they also have physiological and ecological functions that should not be overlooked or underappreciated when assigning them as temporal cues.

The physiological cues inciting adult *V. annecohenae* to become active are likely multidimensional, similar to those observed in DVM. In pelagic plankton, light levels serve as a primary cue (Stearns and Forward 1984; Ringelberg 1999), dictating whether animals move up or down in the water column. Benthic bioluminescent ostracods become active only at low levels of light, which are also required for the initiation of their bioluminescent displays. In migrating plankton, the actual rate of change or magnitude of change in light intensity acts as a secondary cue to determine the timing of movement (Ringelberg 1999). For ostracods, foraging activity and bioluminescent displays peak during the hour following either sunset or moonset, potentially indicating that the ostracods are responding to a secondary cue related to rapid changes in light level.

Peak foraging activity in adult *V. annecohenae* also appears to correspond with times when the maximum numbers of receptive females are present. This pattern supports the dark threshold and reproductive potential hypothesis (Fig. 1d). From life cycle studies in the laboratory, we determined that fifth (A-1) instar female *V. annecohenae* continuously molt into adults and reproductive females constantly release broods over a 24-h period regardless of light levels (Gerrish and Morin 2008). If females constantly become receptive after molting or brood release, and mate only when it is dark, then during extended periods of light reproductively available females will accumulate. Extended periods of light occur during daytime and on moonlit nights, with the longest period of constant light occurring over the 2 or 3 days near full moon when either the sun or a brightly illuminated moon is present in the sky at all times. Thus the first hour of darkness each night and the nights immediately following the full moon would have the highest densities of newly receptive females. These nights also have short windows of time at the beginning of each night when the necessary dark threshold is met (Fig. 1d). Our data indicate that the peak periods of display activity occur during the first 15 min after the dark threshold is reached and then drops off quickly, within less than an hour (Fig. 6). Furthermore, we observed highest foraging activity on the 2 or 3 nights following the full moon. Whether males have adapted to display at these times to maximize fitness or are becoming active due to a pheromone produced by receptive females remains to be tested.

Additional support for the reproductive potential and dark threshold hypothesis is provided by juvenile *V. annecohenae,* which have a much less clearly defined relationship

with darkness. They are nocturnal but appear to respond to a much brighter dark threshold. It is likely that because juveniles are not actively participating in reproduction or bioluminescent displays, their behaviors are less dictated by dark conditions. On the other hand, all age classes and sexes of *V. annecohenae* are capable of emitting bioluminescence and it is postulated to be a means of defense against predators (Morin and Cohen 1991). Assuming that bioluminescence is important in deterring predators, its use as an alarm signal would be hindered on bright nights. One possibility is that the smaller size of juveniles makes them less susceptible to predators that orient visually in moonlit conditions, reducing their dependence on bioluminescent defense. Nevertheless, stage five (A-1) females, which are actually larger than adult males, show a behavioral pattern more like juveniles than adults. They actively feed on moonlit nights when adults do not, strongly indicating that the adult behaviors are due to some correspondence between reproductive and feeding or foraging behavior.

Next to darkness, water velocity is the most important variable influencing foraging by both adults and juveniles. While *V. annecohenae* reach a maximum size of about 2 mm and swim 8–12 cm/s (Rivers and Morin 2008), their swimming and grasping abilities can still be overcome by strong currents. Effects of current are apparent when observing males attempting to display on nights when water velocity is high. The first pulse of a reproductive display occurs in or just at the top of the turtle grass (Rivers and Morin 2008) where the flow is somewhat limited by the grass–water boundary. On nights when water velocity is high we observe one to three bright flashes near the grass and then one or two more flashes above the grass that move swiftly away at the rate and in the direction that the water is moving. Under these conditions, males rarely release more than three to five pulses in a display compared to a typical display in calm conditions of 15–19 flashes (G. A. Gerrish et al., personal observations). Like other bioluminescent ostracod species, *V. annecohenae* males cue off of and entrain on the displays of other males to begin reproductive behaviors (Morin 1986; Rivers and Morin 2009), but this interaction is not evident on high flow nights when displays are limited to three to five flashes. Because adult feeding behavior appears to correspond with reproductive behavior, it is possible that the ostracods experience an overall lack of activity on nights when water velocity is high. This overall lack of activity is supported by the observation that juvenile activity is also suppressed on high flow nights. Because *V. annecohenae* spend most of their time hidden in the sediments, it is possible that they remain submerged to avoid being swept into inappropriate habitats on high flow nights. It is also possible that as scavengers, *V. annecohenae* have difficulty detecting food sources during periods of high flow because the current carries away chemical cues for resource location (Koehl 2006).

The differences observed in feeding activity between January/February and June could indicate seasonal fluctuations in *V. annecohenae* densities. Because quantitative sampling was conducted only during a single January/February and June, we have limited ability to infer seasonal effects. Additionally, environmental conditions, such as temperature and flow varied between January/February and June, confounding a strict temporal inference with the role of environmental drivers influencing *V. annecohenae* densities. The question remains: is seasonal variation driving actual differences in numbers, or are harsher environmental conditions during January/February simply limiting behaviors? Based on Fig. 4a, e, because similar densities of adults were captured in January/February on the few nights when flow was extremely low, we may expect that *V. annecohenae* activity is simply a by-product of environmental factors.

In addition to the measured variables influencing *V. annecohenae* densities that were the focus of this study, microhabitat variation and slight variation in the atmosphere also have the potential to influence *V. annecohenae*. Among the three traps set nightly, we observed high levels of variation in the number of animals captured. Although replicate trap variation was not large enough to prevent our detection of the influences of flow and darkness, there was substantial residual variation. Because of their small size, *V. annecohenae* could be responding to more localized cues within the grassbed habitat.

Overall, our data indicate that the strongest environmental factor influencing the feeding and reproductive behaviors of *V. annecohenae* was the availability of time when illumination was below a critical dark threshold. This dependence on darkness for successful growth and reproduction allows us to classify darkness as a resource, in the same way that others have classified time, space and temperature as resources (Magnuson et al. 1979; Tracy and Christian 1986; Kronfeld-Schor and Dayan 2003). Considering darkness as a resource alters the way we think about important physiological, biological and behavioral activities that take place after the sun goes down. Additionally, it clarifies discussion of the increasing and severe impact that artificial lights have on darkness and hence upon the organisms that depend upon this important natural resource.

Acknowledgments We thank our handling editor Charles Peterson and two anonymous reviewers whose comments helped to improve this manuscript and Suzanne Stapleton for assistance with figure revisions. We also thank Nelson G. Hairston Jr and Amy McCune for comments on project development and this manuscript. Paul Helfenstein was gracious in his assistance with the lunar model. We also are grateful for field assistance from Krystal Rypien, Michael Pipersburgh and

Richie Williams and support from International Zoological Expeditions on Southwater Caye. Funding sources included the College of Agriculture and Life Sciences Cornell University, the Mario Eunaudi Fund and the S. Ann and Robert R. Morley Research Fund. This research was completed in accordance with permits received from the Belize Department of Fisheries, Belize.

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