

Diel activity cycles of freshwater gastropods under natural light: Patterns and ecological implications

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Abstract – Though much is known about freshwater snail ecology, their circadian rhythms remain poorly investigated. Well-fed, stress-free, mid-size adults of six species common in central Italian lakes were exposed to natural sunlight and photoperiod, and their activity status was recorded at 3-h intervals during a 9-d indoor experiment. All species exhibited evident diurnal habits despite high individual variability, with midday-to-early-afternoon activity peaks. Activity was correlated with diel light conditions but not with short-term changes in albedo. The prosobranch *Bithynia* (= *Codiella*) *leachii* and the pulmonates *Physa* (= *Physella*) *acuta* and *Planorbis planorbis* were the most active species and exhibited the longest-lasting response to daytime food addition. The prosobranch *Valvata piscinalis* exhibited long periods retracted in its shell with the operculum shut, and the remaining taxa (the pulmonates *Galba* (= *Lymnaea*) *truncatula* and *Radix* (= *Lymnaea*) *auricularia*) exhibited an intermediate degree of activity. *P. acuta* was the most active species at night and exhibited the quicker response to nighttime food addition. Alertedness to (diurnal) predators may be highest for the highly active *P. acuta* and *P. planorbis*, whose antipredator defenses are mainly behavioral. Diel activity patterns and other ecological characteristics suggest that *P. acuta* may be favored in food-rich habitats, while *V. piscinalis* may not be able to fully exploit food resources, especially if in limiting quantities. All snail species – and *P. acuta* in particular – may stimulate periphyton metabolism while keeping its biomass low by grazing mainly during the time of maximum photosynthesis.

Key words: Circadian rhythms / light irradiance / feeding behavior / herbivory / predation

Introduction

Gastropods are common inhabitants of shallow-water habitats such as lake littoral zones (Jokinen, 1987; Brown, 2001). Freshwater snails are typically herbivores, feeding on periphyton, epiphyton on macrophytes, detritus of plant origin, and occasionally on living macrophyte tissue and decaying fragments of dead invertebrates (Brown, 2001; Lombardo and Cooke, 2002).

Snails can be formidable grazers, continuously “cleaning” the substratum with their radula (Bourassa and Cattaneo, 1998; Muñoz *et al.*, 2000). Physids in particular are capable of exerting top-down control of epi/periphyton even in the presence of strong bottom-up forces (high nutrient concentrations: Jones *et al.*, 1999; Lombardo, 2001). High grazing rates suggest that snails may play a major role in shallow-water habitats, maintaining or promoting high water clarity in eutrophic waters by

enhancing macrophyte well-being through continuous removal of competing epiphyton (Brönmark and Weisner, 1992; Lombardo, 2005). Snail effects on macrophyte productivity may be highest during daylight, when photosynthetic response is highest. However, studies on snail grazing have typically assessed the effects *in toto*, without separating grazing effectiveness or periphyton/macrophyte response in daylight or darkness.

Littoral snails also are typically part of rich and diverse macroinvertebrate communities, which include predators (*e.g.*, Macan, 1977; Lombardo, 2005). Predation on freshwater snails by a number of predators was observed or inferred under controlled conditions (Brönmark and Malmqvist, 1986; Kesler and Munns, 1989; Tripet and Perrin, 1994). However, natural populations of snails tend to co-occur at high density with most of their predators (Macan, 1977; Lombardo, 2005).

Broad-reaching investigations suggest that factors typically absent in narrowly focused laboratory settings

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Table 1. Descriptive characteristics and shell size at t_0 of the six species of gastropods investigated, listed alphabetically. Shell size was taken as diameter for *P. planorbis*, and as height for all other species ($n = 12$ for all).

Species	Subclass: family	Shell size at t_0 (avg \pm std. err., in mm)
<i>Bithynia</i> (= <i>Codiella</i>) <i>leachii</i> Sheppard 1823	Prosobranchia: Bithyniidae	6.8 \pm 0.2
<i>Galba</i> (= <i>Lymnaea</i>) <i>truncatula</i> O.F. Müller 1774	Pulmonata: Lymnaeidae	19.4 \pm 0.9
<i>Physa</i> (= <i>Physella</i>) <i>acuta</i> Draparnaud 1805	Pulmonata: Physidae	11.0 \pm 0.7
<i>Planorbis planorbis</i> L. 1758	Pulmonata: Planorbidae	8.8 \pm 0.2
<i>Radix</i> (= <i>Lymnaea</i>) <i>auricularia</i> L. 1758	Pulmonata: Lymnaeidae	17.4 \pm 0.9
<i>Valvata piscinalis</i> O.F. Müller 1774	Prosobranchia: Valvatidae	4.7 \pm 0.2

may mitigate the potential devastating effects of predators on snails at the community level. For example, some snails are capable of rapid changes in their life histories or shell shape or thickness to minimize predatory losses (Crowl and Covich, 1990; DeWitt *et al.*, 2000; Lakowitz *et al.*, 2008). Using a simplified littoral food web, Lombardo (1997) found that, despite some losses, snails increased in relative abundance thanks to preferential damselfly predation on other prey. Macan (1977) partly explained the conundrum of natural high-density snail–planaria assemblages with planarian low predatory efficiency coupled with high ability to withstand long periods of starvation.

However, other factors potentially contributing to the seemingly stable natural snail–predator coexistence remain largely unexplored. Though recognized – along with food and space – as one of the major niche dimensions (*e.g.*, Pianka, 1976), time remains inexplicably underexplored as a factor potentially separating naturally co-occurring species by means of temporal partitioning of the habitat, or by diel differences in behavior. While some information on temporal habits is available for predators (*e.g.*, Brönmark and Malmqvist, 1986), very little is known about snail diel behavioral patterns that may influence the outcome of predator–snail interactions.

Though circadian rhythms may be disrupted by stress (*e.g.*, McDonald, 1973), the few studies on freshwater snail behavior suggest distinct diurnal, nocturnal, or dawn/crepuscular habits (Beeston and Morgan, 1977; Morgan and Last, 1982; Pimentel-Souza *et al.*, 1984; Rotenberg *et al.*, 1989; see also the review in Dillon, 2000). However, such studies have employed artificial light conditions and/or have focused on economically important species (*e.g.*, intermediate hosts of human parasites). We have determined diel activity patterns for six species of freshwater gastropods common in lake littoral habitats of central Italy, under stress-free, natural-light conditions. The purpose of the investigation was to establish if these species have inherent diurnal or nocturnal habits, and if they partition daily activity temporally.

Materials and methods

Study organisms

The six species of gastropods investigated (Table 1) are common in hardwater, permanent lakes throughout Italy and have a pan-European distribution (Girod *et al.*, 1980;

Bank, 2007). The four pulmonate species are thin-shelled, while the two prosobranchs have a sturdier, thicker shell. All species, except *Bithynia* (= *Codiella*) *leachii*, are hermaphroditic. Sexual dimorphism for *B. leachii* is not apparent. Except for *Valvata piscinalis* which is negatively affected by water temperatures above ~ 23 – 25°C (Burgmer *et al.*, 2007; Mouthon and Daufresne, 2008), all species are adapted to warm (temperature ~ 25 – 32°C), relatively oxygen-poor, and/or eutrophic waters (Berg and Ockelmann, 1959; Girod *et al.*, 1980; Costil, 1994; Ferreri, 1995; authors' personal observations). All species are herbivorous on periphyton and/or detritus (Brown, 2001; Lombardo and Cooke, 2002; Mouthon and Daufresne, 2008); bithyniids and valvatiids may also filter-feed on suspended particles (Kabat and Hershler, 1993; Mouthon and Daufresne, 2008). Nomenclature follows Dillon *et al.* (2002) for *Physa* (= *Physella*) *acuta* and Bank (2007) for all other species.

Experimental snails were randomly picked from long-term laboratory cultures comprising the descendants of individuals originally collected in Lake Ventina (*P. acuta*) or nearby Lake Piediluco (all other species) ($42^\circ 32' \text{N}$, $12^\circ 44' \text{E}$; WGS 84 coordinates) starting from October 2007. The two source lakes are hardwater, meso-eutrophic, seemingly hydrologically connected, and are located in central Italy within the River Tiber watershed (Gaino *et al.*, 2001). Snails naturally co-occurred with invertebrate predators such as leeches and/or dugesiid planarias (Gaino *et al.*, 2001; authors' personal observation). Snails were reared at the Department of Environmental Sciences of the University of L'Aquila in shallow-water, predator- and parasite-free containers with lake water, periphyton-covered coarse-gravel substratum, and macrophyte fragments, all coming from the source lakes. Material from different lakes was kept in separate aquaria. The original lake water in parent aquaria was gradually diluted and eventually replaced with tap water over several weeks. Experimental snails hatched from intact egg clutches that were transferred into clean, tap-water aquaria before hatching to obtain parasite-free and predator-naïve individuals (Dr. Elżbieta Żbikowska, Mikolai Kopernikus University of Toruń, PL, personal communication). Water was replaced weekly to bimonthly to avoid accumulation of toxins, pheromones, or bacteria that may have caused stress and altered snail biology and behavior (*e.g.*, Chaudry and Morgan, 1987). Cultured snails were fed *ad libitum* with natural epi/periphyton and decaying plant fragments, integrated with commercially

Table 2. The four modes used to categorize snail activity, listed from most inactive (top) to most active (bottom). Snails were considered inactive when observed as either inact– or inact+, and active when observed as either act– or act+.

Activity mode	Description
inact–	Absence of any perceived movement and body completely withdrawn into shell with operculum closed shut (prosobranchs), or shell aperture closely adhering to the substratum (pulmonates)
inact+	Absence of any perceived movement, but body not completely withdrawn into shell (head and tentacles partly visible from above); snail apparently “sleeping” or resting, sometimes with production of feces (“digestion”)
act–	Snail in some perceived movement as “act +” (described below), but at a markedly lower degree of movement/activity
act+	Very evident movement (roaming; sliding upside down at the water surface; crawling above water level); active foraging/scavenging with or without locomotion (radular/tentacle movement); oviposition

available lettuce and 5%-*Spirulina*-enriched flake food for aquarium fish to supplement proteins and vitamins. Laboratory-reared snails adapted quickly to fish food, and became able to detect and consume flakes within minutes from addition. Rearing aquaria were placed near a large window facing W, whose adjustable blinds were regulated daily to avoid exposure to direct sunlight and to minimize fluctuations in temperature, which otherwise followed seasonal trends. Cultured snails remained thus exposed to natural daylight and photoperiod since hatching. Species-specific general behavior of laboratory-reared snails did not change appreciably in time, or from field-collected parent individuals. Mortality in all aquaria remained very low through the experiment period.

Experimental setup

The experiment was carried out in an indoor locale in suburban Rome, Italy (41°43'N, 12°21'E). A subsample of several individuals was moved from L'Aquila to Rome in early March to acclimatize to the ambient conditions of the experimental locale. Water temperature, tap water quality, natural photoperiod and irradiance, westerly exposure to natural sunlight, and quantity and quality of food provided were similar in the long-term culturing locale in L'Aquila and in the pre- and experimental locale in Rome. Fifteen-to-eighteen adult individuals of each snail species were randomly picked up from the Rome subsample aquaria 24 h before the beginning of the experiment, transferred into a small container with ~1 L of tap water, and transported to the experimental locale, where they were left to reach ambient temperature overnight in moonlit, natural darkness. Snails were fed a mixture of lettuce leaves, periphyton, and commercial fish flake food *ad libitum*.

The next morning, 72 glass jars were each filled with 125 mL of tap water and placed in 6 rows × 12 columns on a white-surface desk alongside a large unobstructed window facing WNW. Jars received diffuse natural daylight from dawn through dusk. Midday light irradiance at the jar water surface was ~70–120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, simulating natural conditions in vegetated lake littoral zones at ~1 m of depth (P. Lombardo, unpublished data). Jars had been analytically cleaned before use with a 10% HCl

solution, followed by thorough rinsing with tap water, to eliminate preexisting chemical cues that may have influenced snail behavior. Tap water in the jars was left undisturbed for ~14 h to lose excess chlorine and reach an equilibrium with ambient temperature. Snails were starved during this period. Water temperature in jars and in the snail-containing aquarium reached an equilibrium at ~19.6 °C at ~22:00.

At this time, twelve mid-size adults of each snail species (regardless of sex for *B. leachii*) were randomly picked from the transporting container and placed in the experimental jars following a modified Latin-square layout, in which each six-jar column (perpendicular to the window) was assigned randomly within each of two contiguous Latin squares, so that each square of 6 × 6 jars featured one individual of each taxon per row and per column. Such a design allowed to equally distribute any small between-row difference in light irradiance among the six snail species. Each jar received one snail individual, which was initially placed at the jar center to standardize initial conditions. The remaining snails were left in the transporting container near the experimental jars, to monitor snail well-being and behavior under parallel, non-experimental conditions.

The activity of each snail individual was recorded following the scheme in Table 2 every 3 h starting from 0:00 (midnight) on 16 March (*i.e.*, ~2 h after snail addition to jars) through 24 March 2009, spanning nine consecutive 24-h cycles. The degree of activity (act– and act+) was relative to each species, so that individuals of species naturally more active *sensu lato* (*e.g.*, *B. leachii*, *P. acuta*) were proportionately more active than individuals for other species in the same activity mode; for example, act+ *P. acuta* individuals were, on average, more active (*e.g.*, mobile) than act+ *V. piscinalis* individuals. Inactivity was defined as absence of any detectable body movement (including tentacle or radular movements) during 10–20 s of close visual inspection. Preliminary inspections lasting >30–40 s, especially at night, appeared to startle and “wake up” inactive snails and were avoided. Nighttime observations were made with the help of a small flashlight covered with a dark-red semitransparent plastic filter to minimize disturbance (Peckarsky and Cowan, 1995; Tomba *et al.*, 2001). Records for individuals which died during the 9-d investigation were excluded since

3–6 observation rounds before death; such individuals were thus maintained as replicates, but their activity data were averaged over a lower number of daily cycles.

Shell size of each snail individual was recorded at the beginning (t_0) and end (t_F) of the investigation with an electronic precision caliper (instrument error = 0.01 mm). Shell size was determined as shell diameter for *Planorbis planorbis*, and as shell height for all other species. The number of egg masses produced by each individual was recorded at t_F . Surviving snails were returned to the culturing aquaria at the end of the experiment.

Each 3-h round of observations began with recording snail activity, avoiding any physical contact with the jars and direct flashlight beam (at night) on individual snails to prevent any influence on behavior, followed by determinations of water temperature and pH, and light irradiance at the jar water surface. In the few cases when indirect-beam flashlight was insufficient to discern snail behavioral mode reliably, the beam was oriented away from yet-to-be-observed individuals, and the activity mode observed in the first 2–4 s was recorded: inactive snails disturbed by a direct flashlight beam in preliminary trials appeared to “wake up” after 4–5 s; snails active at night did not change their behavior appreciably for the first 15–20 s after being hit by direct flashlight beam.

Physicochemical variables were determined with electronic equipment at one randomly chosen jar for each of three subblocks of contiguous jars (4 window-parallel rows \times 6 columns). Water temperature was determined with a NIST-calibrated Traceable[®] minithermometer (instrument error = 1 °C), and pH was determined with a handheld pH-meter calibrated at circumneutral values at 20 °C (instrument error = 0.1 pH units). The small submersible probes minimized physical disturbance during reading. Water temperature and pH remained within the relatively narrow ranges of ~16–20 °C and 7.9–8.3 units, respectively. Such ranges are unlikely to influence the biology of any of the snail species investigated (Hodasi, 1976; Girod *et al.*, 1980; Costil, 1994; Mouthon and Daufresne, 2008; and authors' personal observations); therefore, water temperature and pH are not treated further in this study.

Light irradiance was determined with a sunlight-calibrated aerial quantum meter (instrument error <1%), measuring photosynthetically active radiation (PAR). The light meter was held just above the jars at ~45° facing the window. Light:dark conditions followed the natural daylight cycle, around spring equinox (D:L ~12:12 h). The 6:00 and the 18:00 observation rounds corresponded to dawn and dusk conditions, respectively. Each complete round of observations and measurements was carried out in ~12–15 minutes.

Food was added at regular 36-h intervals since 0:30 on day-1 ($d_1 = t_0$). The 36-h interval allowed to have three alternating daytime and nighttime food additions, thus avoiding food-induced bias in diel activity patterns. Food consisted of a mixture of periphyton and fish food in flakes. The quantity of food added at each event was designed to allow leisurely feeding for the first ~24 h and

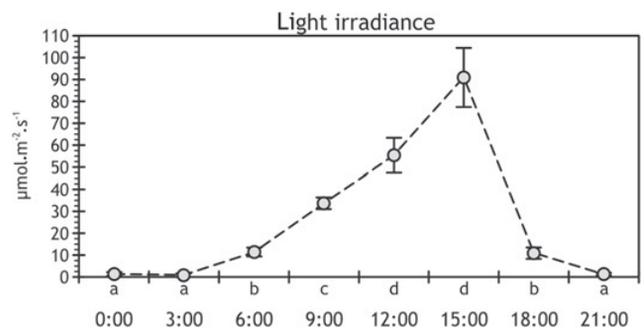


Fig. 1. Light irradiance during the 24-h observation cycles, with observations carried out every 3 h starting at midnight (average \pm standard error; $n = 9$ for each time period). Lower-case letters identify different average values according to an SNK test ($p \leq 0.05$) performed after a significant one-way, type I ANOVA on log-transformed data ($F = 103.175$, $df = 7,64$, $p < 0.001$).

a brief 12-h period of starvation comparable for all species, and was thus tailored to each species based on preliminary trials. The 12-h starvation period was designed to avoid excess leftover that may have led to bacterial development in the jars, and to mildly stimulate snail response to the next feeding event, based on information in Ter Maat *et al.* (2007). Response to food inputs was determined as changes in activity at 15-min intervals from just before food addition (at 12:30 or 0:30) through the next scheduled observation round. Food-addition events were started 30 min after a regular observation round because snails were observed regaining their original activity mode within 10–20 min from mild disturbance in preliminary trials; the 30-min hiatus therefore allowed to have undisturbed snails at the first (pre-feeding) observation event. Individual snails were recorded only as active or inactive during the food-response observations to allow faster inspection rounds (~10 min each). Food addition did not cause appreciable alterations in pH.

Statistical analysis

Analysis was based on the times of occurrences in any given mode of each snail individual averaged over the 9-d experimental duration. The single value per individual obtained this way was thus a true replicate, avoiding the issue of temporal pseudoreplication (*sensu* Hurlbert, 1984) that may stem from multiple observations on the same individuals. Taxon-specific analysis employed one-way, type I ANOVAs on such true replicates to test for differences among observation times. Such a method is deemed more robust than an otherwise equivalent nested design, in which individual daily observations would be kept as subreplicates (Gotelli and Ellison, 2004). Data were expressed as percent of total number of individuals, so that data transformation was not necessary (Zar, 1998). Significant ANOVAs were followed by

Table 3. Numerical characteristics of the six gastropod species at t_F . Shell size is as described in Table 1. For determinations of average values, n is given in the column at the left; nc = not calculable. Number of individuals per species at t_0 was 12.

Species	Number of surviving individuals at t_F	Increase in shell size at t_F (avg \pm std. err., as % of size at t_0)	Number of egg-laying individuals by t_F	Number of egg masses laid per egg-laying individual (avg \pm std. err.)
<i>B. leachii</i>	12	2.8 \pm 1.4	7	2.4 \pm 0.3
<i>G. truncatula</i>	12	2.3 \pm 0.4	2	2.0 \pm 0
<i>P. acuta</i>	12	1.9 \pm 0.4	11	4.7 \pm 0.7
<i>P. planorbis</i>	12	6.8 \pm 0.5	9	3.3 \pm 0.6
<i>R. auricularia</i>	9	1.5 \pm 0.7	5	1.8 \pm 0.4
<i>V. piscinalis</i>	12	4.8 \pm 1.4	1	4.0 \pm nc

Student-Newman-Keuls (SNK) multiple-comparison tests with $p \leq 0.05$. Two-tailed paired t -tests were used to quantify the degree of activity (act– + act+) at taxon level; paired tests were used to bypass the potential statistical shortcomings caused by the negatively correlated data (Underwood, 1997).

Species-specific peak activity times were calculated as average angles on angle-transformed hourly data [$x' = \frac{(360) \cdot x}{24}$] with associated coefficients of angular concentration (r_c) (Batschelet, 1965, 1981; Zar, 1998); differences were tested with a second-order analysis of angles (Hotelling, 1931) as modified by Lombardo and Cooke (2004). Species-specific daily peak activity times significantly different from the group's average were determined as nonoverlapping peak time \pm pooled standard error (Lombardo and Cooke, 2004). Angular statistics proved unreliable for incomplete-cycle food addition data and were applied only to complete 24-h cycle data. Graphical rendition of diel data remained linear for clarity purposes.

Temporal changes in light irradiance were detected with a one-way, type I ANOVA followed by an SNK test ($p \leq 0.05$) on log-transformed data (Bartlett's formulation: $x' = \log_{10}(x + 1)$). Correlations between selected datasets used untransformed data because of analysis reliability when nonnormality is not extreme (Zar, 1998). For correlations, independence of activity data at subsequent 3-h observation times (e.g., 12:00 and 15:00) was assumed based on the much-shorter activity bouts observed for all species in preliminary and experimental trials; feeding events were not used in correlations because of evident autocorrelation between subsequent 15-min-spaced observation times.

Results

Light irradiance at the water surface exhibited evident day–night cycles, with full statistical separation among the three full-daylight (9:00, 12:00, and 15:00), the three full-nighttime (21:00, 0:00, and 3:00), and the twilight observation rounds (6:00 and 18:00) (Fig. 1). Weather conditions were variable but overall benign; for example, conditions at 15:00 ranged from cloudless or mostly sunny skies (five events) to partly cloudy (three events) to overcast and light rain conditions (one event). All snail species grew slightly and laid eggs, and all but three

R. auricularia individuals survived through the 9-d experiment (Table 3).

All species exhibited the highest degree of activity during daytime (SNK separation in Fig. 2). *B. leachii*, *P. acuta*, and *P. planorbis* exhibited the highest daytime activity, with ~ 70 – 80% of individuals recorded as active at peak activity times. These species also tended to be significantly active during full daytime (9:00 through 15:00), significantly inactive in full nighttime (21:00 through 3:00), and at an intermediate degree of activity in twilight (6:00 and 18:00) (paired t -tests in Fig. 2). All species except *R. auricularia* were significantly active at 12:00 and all were significantly inactive at 3:00 (marginally so for *P. acuta*). *R. auricularia* never reached a significant level of activity (paired t -tests in Fig. 2).

Pulmonate snails and *B. leachii* were rarely observed fully retracted in their shells (i.e., mode inact–), while the operculate prosobranch *V. piscinalis* spent considerable time in such mode (Fig. 2). Despite the high individual variability, *V. piscinalis* was always observed significantly more frequently in mode inact– than all other species (SNK separation at $p \leq 0.05$ after significant one-way, type I ANOVAs for each time interval, with $F \geq 3.668$ and $p \leq 0.004$; $df = 6, 48$ for all). All species were often observed sliding upside down at the water surface (one of the active modes described in Table 2), and all pulmonates were observed crawling above the water surface, often staying there in mode inact+ for some time, especially after food additions. Such individuals reentered the water medium shortly thereafter.

Snail activity was significantly, positively correlated with diel light irradiance for all species, though the level of significance was marginal for *Galba truncatula* (Table 4). However, activity was not correlated with light conditions during the periods of highest irradiance and/or highest degree of activity (12:00 and 15:00).

The six gastropod species collectively exhibited an early-afternoon (12:54) daily peak in activity, but peak activity times differed significantly among the six gastropod species (Hotelling's (1931) second-order analysis of angles: $F = 17.588$, $p = 0.011$) (Fig. 3). *B. leachii*, *P. planorbis* and *R. auricularia* shared similar daily peak activity times, while *P. acuta*'s peak activity at 14:02 was the most (significantly) distinct from the group average. Coefficients of angular concentration (an inverse measure

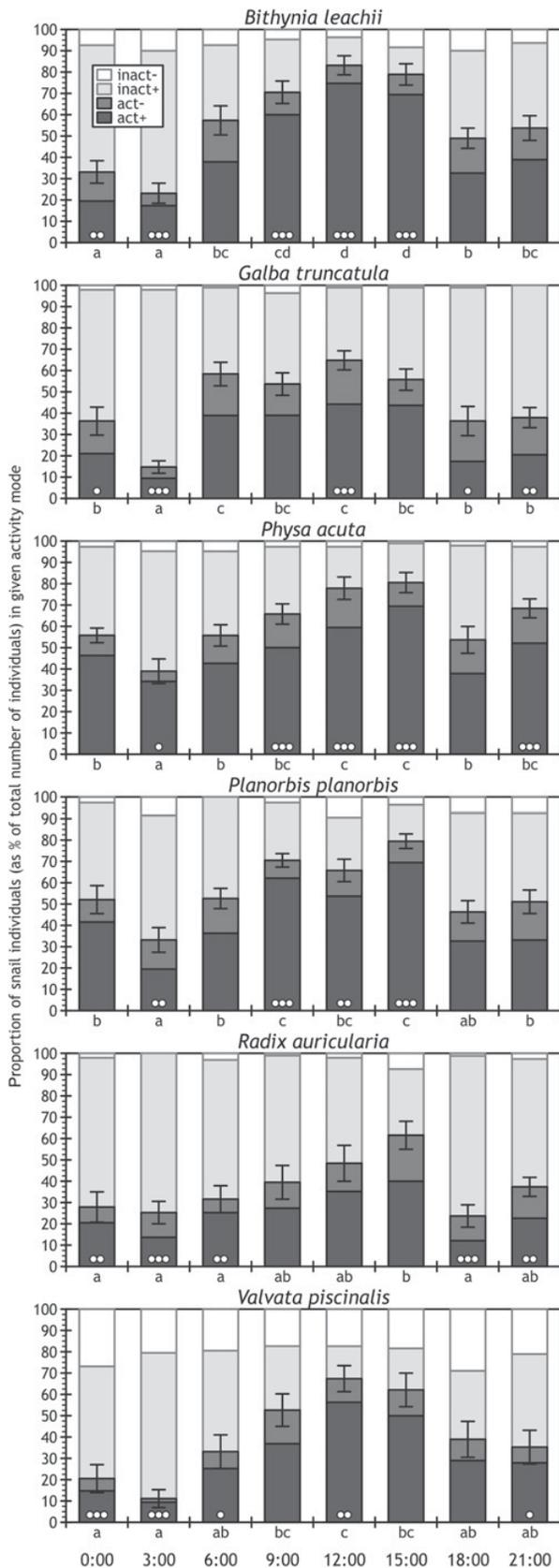


Fig. 2. Average individual activity cycles, as proportion of occurrence in the given mode by 3-h observation interval, for the six species examined; $n = 12$ for each species in each time period. Standard error and statistical analysis are given for total “active”

of variability) were low for all species ($r_c = 0.11\text{--}0.31$, Fig. 3).

Daytime food addition was associated with an increase in the level of activity only for *P. planorbis* (SNK separation in Fig. 4). *B. leachii* and *P. acuta* maintained a highly significant degree of activity through the daytime food addition, while *G. truncatula* eventually slowed down to nonsignificant activity levels by the next observation round (paired *t*-tests in Fig. 4). *R. auricularia* exhibited the slowest average response to daytime food addition ($\sim 2\frac{1}{2}$ h after food addition, as significant degree of activity from nonsignificant values for the paired *t*-tests in Fig. 4). Nighttime food addition caused an increase in the degree of activity (paired *t*-tests) for all species, either from significantly inactive to nonsignificant (*B. leachii*, *G. truncatula*, *R. auricularia*, and *V. piscinalis*) or from nonsignificant to significantly active (*P. acuta* and *P. planorbis*), though the increase was strong only for *G. truncatula* and *P. acuta* (SNK separation in Fig. 4). *P. acuta* exhibited the quickest response to nighttime food addition (SNK separation after significant ANOVAs at each 15-min time interval; detailed results not shown). Nighttime activity was restored to pre-feeding levels $\sim 1\frac{1}{2}$ –2 h after food addition for most species.

Response time of inactive (inact– + inact+) snail individuals to food additions varied from ~ 15 (*P. acuta*) to ~ 165 min (*V. piscinalis*), but between-species differences remained qualitative (one-way ANOVAs on log-transformed data: $F_{\text{day}} = 0.262$, $p = 0.93$, $df = 5,44$; $F_{\text{night}} = 1.231$, $p = 0.31$, $df = 5,62$), as did species-specific daytime vs. nighttime differences (two-tailed *t*-tests performed separately for each species using log-transformed data: $t = 0.229\text{--}1.010$, $p = 0.33\text{--}0.82$, $df = 14\text{--}21$). However, nighttime response of inactive snails to food addition was significantly slower than daytime response when data were pooled (paired *t*-tests using log-transformed average values for each species: $t = 5.342$, $p = 0.003$, $df = 5$). When fully retracted inside their shells with the operculum shut, *B. leachii* and especially *V. piscinalis* seldom responded to food additions within the $2\frac{1}{2}$ -h observation periods, while the individual response of inact– pulmonates was more variable; however, this behavior was not quantified.

Discussion

Experiment-long shell growth and oviposition (Table 3) recorded for all species suggest that the

mode (= act– and act+); lower-case letters identify different average values according to SNK tests ($p \leq 0.05$) performed after significant one-way, type I ANOVAs. Significant differences between proportions of active (act– + act+) and inactive occurrences (inact– + inact+), tested with two-tailed paired *t*-tests, are given at three levels of significance ($\circ = p < 0.10$, $\circ\circ = p < 0.05$, and $\circ\circ\circ = p < 0.01$). Activity modes are described in Table 2, and detailed statistical results are in Tables A1 and A2 (Online Material available at www.limnology-journal.org).

Table 4. Linear regressions between light irradiance and snail activity. For 24-h cycle analysis, average light irradiance was regressed against average activity (as % of individual occurrences in active mode) for each of the 3-h-spaced observation events ($n = 8$); analysis of the highest light irradiance used daily values at 12:00 and 15:00 ($n = 18$). For all regressions, $df = n - 2$.

Species	r^2	Average values, 24-h cycles			r^2	Daily values, 12:00 and 15:00	
		p	Trend	p		Trend	
<i>B. leachii</i>	0.661	0.01	+	0.099	0.20	+	
<i>G. truncatula</i>	0.421	0.08	+	0.111	0.18	–	
<i>P. acuta</i>	0.626	0.02	+	0.103	0.19	+	
<i>P. planorbis</i>	0.755	0.02	+	0.043	0.41	+	
<i>R. auricularia</i>	0.902	<0.01	+	0.002	0.87	–	
<i>V. piscinalis</i>	0.714	0.01	+	0.099	0.20	+	

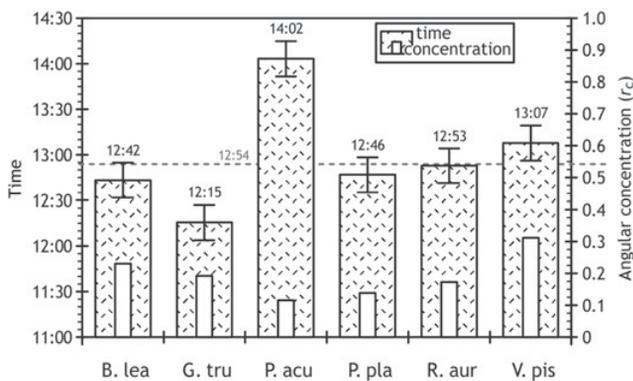


Fig. 3. Daily peak activity times for the six gastropod species, calculated as average angular data \pm pooled standard error (left axis). The angular concentration (r_c , right axis) is a measure of species-specific variability in behavioral activity, ranging from zero (maximum variability) to one (absence of individual variability) (after Zar, 1998). The horizontal dashed grey line identifies the group peak time (12:54) averaged over the six species (as angular grand average of species-specific averages, after Zar, 1998). Daily peak activity times \pm pooled standard error not overlapping with the average group peak time of 12:54 differ significantly from the group average (after Lombardo and Cooke, 2004). Taxa are identified by genus initial followed by the first three letters of the species name; full names are in Table 1.

experimental conditions, including the 36-h food additions with the deliberate \sim 12-h pre-feeding starvation period, were not stressful. Based on qualitative observations in the 1-L “leftover” container and in culturing aquaria, the low degree of oviposition of *G. truncatula* and *V. piscinalis* (Table 3) appeared to be associated with seasonal reproductive cycles. Absence of jerky movements by active snails, rare long-term wandering above the water line by pulmonates, and rare complete withdrawals into shells also indicate minimal or absent stress (Turner *et al.*, 1999; Hourdin *et al.*, 2006; Ms. Sarah Rid, University of Konstanz, personal communication). Short-term crawling just above the water level, with snails voluntarily reentering the jar water by the next observation round, appeared to be most often post-feeding moments of rest, and was not interpreted as a response to stress. Based on general behavior in culturing and leftover aquaria, the sometimes several hours spent by *V. piscinalis* without any movement

and fully retracted in their shell (inact– mode) appeared to follow long activity periods (spent mainly foraging), and were similarly not interpreted as a sign of stress. Experiment-wide mortality was limited to three *R. auricularia* individuals, also supporting the interpretation of general absence of stress in the experimental jars. The cause for the three *R. auricularia* deaths remains unknown, as such individuals did not behave unusually or differently from fellow *R. auricularia* individuals until shortly (*i.e.*, for 2–5 observation rounds) before their deaths, and we remain unable to ascertain any cause–effect relationship between the low degree of activity (Fig. 2) and the experiment-related mortality of *R. auricularia*.

All six species exhibited evident diurnal habits (Figs. 2 and 3, Table 4), supporting the view that stress-free freshwater snails follow distinct, species-specific diel or dark–light activity cycles (*e.g.*, Beeston and Morgan, 1977; Pimentel-Souza *et al.*, 1984). Light was proven or inferred as a strong behavioral clue also in earlier investigations (Pimentel-Souza *et al.*, 1984; Rotenberg *et al.*, 1989; Ter Maat *et al.*, 2007). However, absence of a fine-scale correlation between activity and light irradiance during the daily period of highest activity and irradiance (Table 4) suggests that short-term changes in albedo do not influence snail diel cycles. All species also exhibited high individual variability (*e.g.*, low r_c values in Fig. 3), suggesting variability in individual conditions (*e.g.*, hunger) and/or a high degree of phenotypic plasticity, supporting earlier results (Russell-Hunter, 1961; Perrin, 1986; Cowl and Covich, 1990; Lakowitz *et al.*, 2008). Despite high individual variability, all species (except *R. auricularia*) were significantly active for at least one time interval at or around noon (significant paired *t*-tests in Fig. 2).

Independent, qualitative parallel observations in culturing aquaria and in the “leftover” small container suggest that most species feed during daytime through the early evening hours, then rest apparently digesting (production of feces) under or above the water level for a short time, before entering a short-lived “second wind” time of activity around midnight in which they seek sexual mates, eventually entering an apparent “deep sleep” by \sim 1:30–2:00. Such a qualitative behavioral pattern is reflected in the experimental results of a midday-to-afternoon peak in activity (Figs. 2 and 3) followed by a period of “rest” at dusk and/or into the early evening

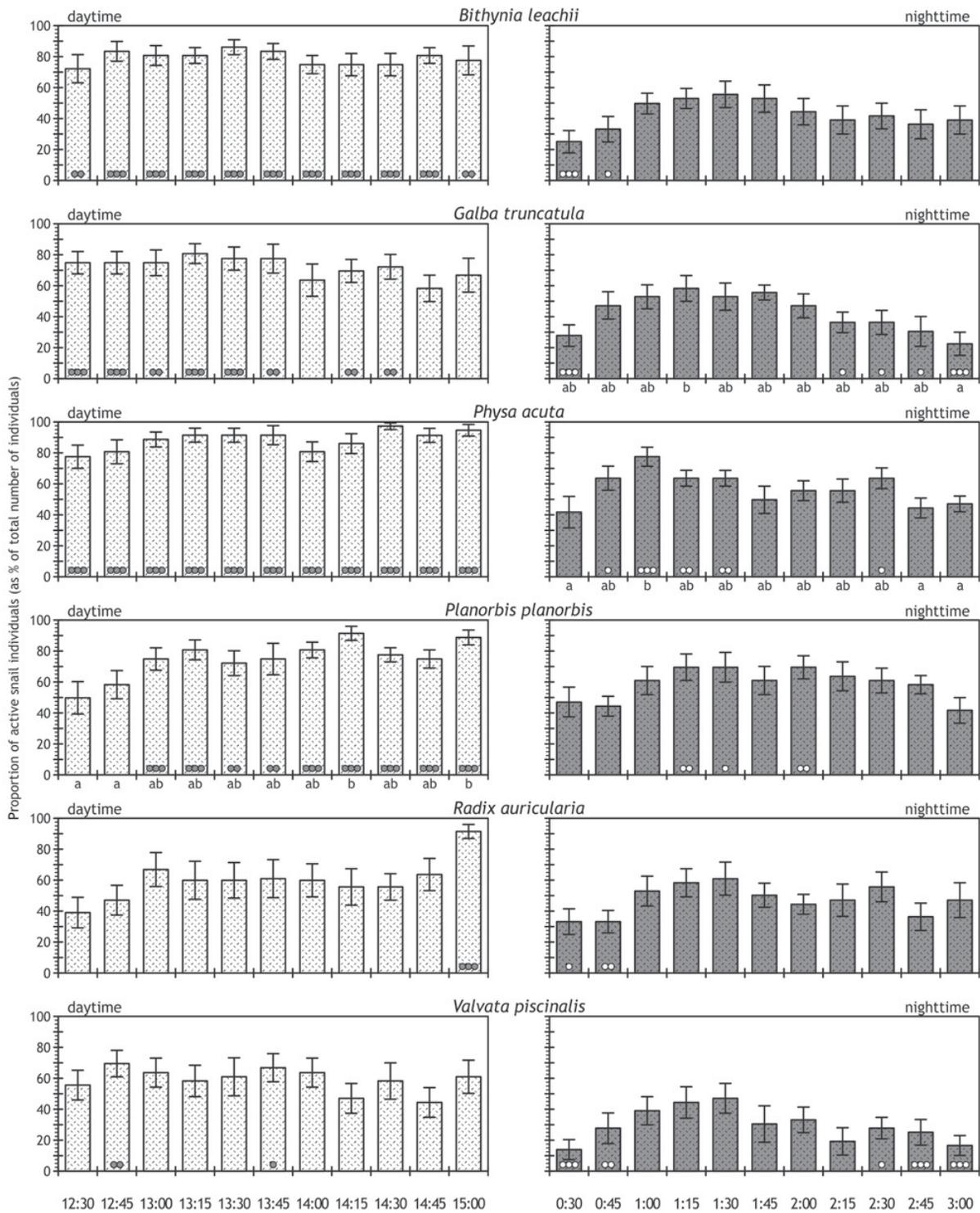


Fig. 4. Occurrence in active mode (as % of total number of individuals; average \pm standard error) just before (12:30 or 0:30) and at 15-minute intervals after daytime (left panels) and nighttime food addition (right panels), for the six species examined. Other explanations as in Figure 2; detailed statistical results are in Tables A3 and A4 (Online Material).

hours, followed in turn by a reprise in activity at midnight before entering the quantitative moment of highest diel inactivity at 3:00 (SNK separation in Fig. 2).

Response to daytime food addition remained mostly nonsignificant, either because most species were already highly active just before food addition (*B. leachii*,

G. truncatula, *P. acuta*), or because individual response to food addition was highly variable (*R. auricularia*, *V. piscinalis*), resulting in nonsignificant statistical outcomes (*t*-tests and/or SNK separation in Fig. 4; between-species SNK separation at $p \leq 0.05$ (not shown)). The species that were most active during daytime

(*B. leachii*, *P. acuta*, and *P. planorbis*) also remained highly active throughout the 2½-h daytime food event period (Fig. 4), suggesting that these species may readily take advantage of food, whether the latter is found *via* active pursuit or provided as pulse inputs (*e.g.*, detritus or macrophyte fragments carried by water currents). *P. acuta* exhibited the quickest and most intense response to nighttime food addition, both as a significant increase in average activity within 15 min from food addition, and as average number of active individuals (~78% of total number of individuals) within 30 min from addition (*t*-tests and SNK separation in Fig. 4 and for between-species comparisons; detailed results not shown). General activity patterns and response to food suggests that *P. acuta* may be the most opportunistic species among the six investigated, being capable of quickly exploiting food sources at any time of the day. However, the short-lived and/or mild response to nighttime food addition exhibited by most species (Fig. 4), coupled with the “community-wide” significantly longer nighttime response ($p = 0.004$ for a paired *t*-tests using pooled data), strongly support the general diurnal activity patterns displayed by all species (Figs. 2 and 3).

The activity patterns found in this study may reflect broader species-specific ecological characteristics which, in turn, may be related to community-level features such as interspecific competitive ability. For example, *P. acuta* is considered a poor competitor (Brown, 1982; Perrin, 1986; Jokinen, 1987) despite its high grazing effectiveness (*e.g.*, Lowe and Hunter, 1988; Lombardo, 2001; but see Wojdak and Mittelbach, 2007) and success in productive habitats at both local/regional (*e.g.*, Girod *et al.*, 1980, and authors’ personal observations) and global scale (Dillon *et al.*, 2002). *P. acuta*’s high activity and quick response to food may translate into a high potential to exploit food, which may be exacerbated by its fast way of moving around (thus increasing access to food patches: Lombardo and Cooke, 2004). Similarly, *P. acuta*’s high individual variability and daily activity peak significantly later than all other species (Fig. 3) may alleviate some of the competitive pressure by reducing interference-based interactions with co-occurring snail species. Though the hypothesis remains untested, such features may thus compensate for *P. acuta*’s inherent poor competitive ability at least in food-rich (and hence often species-rich; *e.g.*, Jokinen, 1987; Costil and Clement, 1996) gastropod communities, which typically feature abundant physid populations (*e.g.*, Jokinen, 1987; Lombardo, 2005). The high degree of activity also may be behind the apparent replacement of the native *Physa fontinalis* L. 1758 by the North American *P. acuta* in much of western and Mediterranean Europe (Anderson, 2003; Cianfanelli *et al.*, 2007; García-Berthou *et al.*, 2007); however, direct comparisons between these two physids are needed to support this hypothesis.

General low diel activity and related behavioral patterns of *V. piscinalis* (*e.g.*, high degree of time spent with the operculum closed shut; short-lived response to nighttime food addition: Figs. 2 and 4) also suggest

poor competitive abilities, a hypothesis consistent with Mouthon and Daufresne’s (2008) view that this species has failed to recover from acute thermal stress because of high competition for post-disturbance limited food resources. Low *in situ* abundance of the otherwise common *V. piscinalis* in central Italian lakes (Mastrantuono and Mancinelli, 2005; authors’ personal observations), may be similarly related to poor competitive abilities, but specific studies are needed to verify this hypothesis.

Though it spent considerable less time in mode inact–than *V. piscinalis*, *B. leachii* also exhibited a poor (if any) response to food addition when in such mode, suggesting that the lowered competitive abilities resulting from such an “isolationist” behavior may be a trade-off for an effective antipredator defense. *B. leachii*’s *de facto* invulnerability to predatory attacks by invasive invertebrate predators such as dugesiid planarias in specifically designed experiments (P. Lombardo, unpublished data) supports this hypothesis (*V. piscinalis* was not tested), as do earlier findings of a combination of operculum shutting and chemical predator recognition for *B. tentaculata* and/or *V. piscinalis* as an effective defense against leech predation (Brönmark and Malmqvist, 1986; Kelly and Cory, 1987). In general, active diurnal species may be more alert against diurnal predators and more vulnerable to nocturnal predators. Such a hypothesis is consistent with the predominantly behavioral antipredator defenses displayed by the most active, but structurally vulnerable, pulmonates tested in this study (*P. acuta* and *P. planorbis*) (*e.g.*, Turner *et al.*, 1999; but see DeWitt *et al.*, 2000). *B. leachii* instead may resort to behavioral responses when active (*e.g.*, by quickly retreating into the shell and shutting the operculum: Brönmark and Malmqvist, 1986) and to structural defenses when inactive (*i.e.*, thick shell with operculum closed shut). The overall higher degree of activity, including at night (Fig. 2), exhibited by the otherwise structurally vulnerable *P. acuta* supports the hypothesis that this species strongly relies on behavioral antipredator defenses that require a certain degree of constant alertness (*e.g.*, crawling out of water, detaching from substratum, *etc.*: Turner *et al.*, 1999). However, studies specifically targeting a link between activity and antipredator defense are needed to test this hypothesis.

Their inherent diurnal habits may make the six species tested in this study particularly effective at shortcircuiting energy and nutrients within the benthic component of littoral food webs, while maintaining low standing crops of periphyton. In fact, periphyton heavily grazed upon by snails often responds with increased productivity despite sustained low standing crop (Bourassa and Cattaneo, 1998; Muñoz *et al.*, 2000). Nutrient uptake by algae is higher in the light, with some leaking back into the water near dusk (Overbeck, 1962; Soeder, 1965). The consequent sustained demand for nutrients would retain much of the latter within the benthic compartment of the littoral zone, preventing phytoplankton from developing and thus maintaining high water clarity in the littoral zone. Periphyton is also favored over macrophytes for nutrient uptake from the water column (*e.g.*, Moeller *et al.*, 1988),

so that low periphyton biomass on macrophytes allows the latter to take full advantage of light and dissolved nutrients (Jones *et al.*, 2000; Lombardo, 2005), allowing for a healthy, dense growth. Healthy macrophytes in well-lit, nutrient-rich waters also may further inhibit phytoplankton by producing more anti-algal allelochemicals (Gross, 2003). Predominantly diurnal grazing on periphyton may then translate into higher periphyton and macrophyte productivity, as autotrophs would be stimulated at the time of maximum photosynthetic rate (thus accelerating nutrient recycling within the littoral, benthic community), but into higher biomass only of unpalatable macrophytes. Because macrophytes are central to sustained water transparency in shallow-water, nutrient-rich habitats (*e.g.*, Scheffer, 1998), heavy periphyton grazers with diurnal habits may effectively contribute to stabilize a macrophyte-dominated clear-water state even in otherwise phytoplankton-favoring nutrient-rich habitats. Physid commonness, high effectiveness as periphyton grazers (Jones *et al.*, 1999; Lombardo, 2001), and highly active (feeding) behavior (this study), suggest that *P. acuta* may indeed play a keystone role in macrophyte-based littoral food webs, as suggested by Lombardo (2001), and more so than other snail species with similar diurnal habits.

Other aspects remain untested. For example, we tested individual snails in isolation, and the influence of neighboring conspecifics (or allospecifics) on individual behavior remains unknown. This aspect may be particularly important for *P. acuta*, given its more flexible habits and quicker response to stimuli (Figs. 2–4) coupled with its typical occurrence in crowded populations and communities (Jokinen, 1987; Lombardo, 2005; authors' personal observations). Increases in activity following external stimuli (Fig. 4) suggest that *P. acuta* may be more active in multiple-individual communities than in isolation. If *P. acuta* in naturally crowded communities is indeed more behaviorally flexible and responds more quickly to stimuli than in isolation, its potential role as a keystone grazer in littoral communities and its anti-predator behavioral “sensitivity” may be even more pronounced than our investigation suggests. Also, the activity scale used in this investigation (Table 2) was “standardized” across species, so that the effective degree of activity-related grazing pressure for the highly mobile, “fast” *P. acuta* may be even higher than this study suggests. In order to fully assess the ecological role of *P. acuta* and other freshwater snails in natural communities, we advocate more studies addressing the potential influence of temporal aspects on snail behavior and ecological characteristics.

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