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Fecundity and Growth Rates in a Cave-Dwelling Population of *Physa acuta* (Gastropoda, Basommatophora, Physidae) Under Simulated Cave and Surface Conditions

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ABSTRACT

We conducted experiments comparing growth rates and fecundity of Stemler Cave *Physa acuta* reared under simulated cave and simulated surface conditions, using nutrient-poor well water and nutrient-rich cave water. Average egg production rates by parent snails were dramatically lower under cave conditions. Hatchling snails reared under surface conditions had higher growth rates than snails reared under cave conditions and snails reared in cave water had higher growth rates than snails reared in well water. Surface treatment snails raised in cave water had the overall highest growth rate, while cave treatment snails raised in well water had the lowest average growth rate. Surface treatment snails reared in cave water were the first to reach sexual maturity, producing viable embryos 32 days post-hatching. This study provides life history data that could help assess the potential for *Physa acuta* to compete with the state endangered Enigmatic cavesnail (*Fontigens antroecetes*) in Stemler Cave.

Key Words: fecundity, growth rates, phenotypic plasticity, *Physa acuta*, Stemler Cave

INTRODUCTION

The caves and groundwater of the sinkhole plain karst (SHPK) of southwestern Illinois, USA are contaminated with a variety of agricultural and household pollutants (Dodgen et al., 2017). Nutrient enrichment in the SHPK has created the potential for troglophilic and surface aquatic species to outcompete stygobitic (obligate cave-dwelling) species in caves (Taylor and Webb, 2000, Sket, 1999). Bioinventories of SHPK caves show widespread occurrence of physid snails whose taxonomic relationships are unresolved and whose life histories in are essentially unknown (Hubricht, 1941, Peck and Lewis 1978, Lewis et al., 2003). Lewis et al. (2003) recorded a troglophilic/troglobitic physid in 16 SHPK caves (including Stemler Cave, St. Clair County, Illinois) and considered all to be the same taxon (*Physa* sp.). Fogelpole Cave in Monroe County, Illinois is home to a polymorphic population of snails, tentatively identified as *Physa gyrina*, with several pigmented and albino morphotypes (Weck and Taylor, 2016). The *Physa* found in Stemler Cave are pigmented and show no obvious troglomorphic characteristics (Figure 1). Dr. Stephanie Clark (personal communication, 23 October 2012) positively identified the Stemler Cave population as *Physa acuta* Draparnaud, 1805, a snail known for its ability to invade new habitats (Dillon et al., 2006). Voucher specimens of Stemler Cave *P. acuta* have been deposited in the collections of the Field Museum of Natural History, Chicago, Illinois, USA (FMNH 329765 and FMNH 329766).

In Stemler Cave, *Physa acuta* is syntopic with the Illinois state endangered Enigmatic Cavesnail, *Fontigens antroecetes* (Hubricht, 1940), a stygobitic species whose Illinois distribution is restricted to Stemler Cave. Life history studies conducted under simulated cave conditions revealed that *F. antroecetes* has long embryonic development times, slow growth rates, and long maturation times typical of stygobionts (Weck, 2022). The purpose of this study is to gain information about the growth rates and reproductive capacity of the Stemler Cave population of *P. acuta* under simulated cave and surface conditions, providing information that could be useful in determining the potential for *P. acuta* to compete with the *F. antroecetes* in Stemler Cave.

Figure 1. *Physa acuta* from Stemler Cave (St. Clair County, Illinois, USA). Photograph by Derik Holtmann.

METHODS

Two adult *P. acuta* were collected from Stemler Cave on 23 August 2013 and reared in the lab following the protocol of Dillon and Wethington (1992) to establish a colony that was maintained for one year. We used a two-factor experimental design to test the effects of nutrients in the water (high nutrient cave water vs. low nutrient well water) and temperature + light combined (simulated cave vs. simulated surface conditions) on snail growth rates, fecundity, and embryonic development. Cave water was collected directly from Stemler Cave and filtered with a 0.45-micron filter to remove large particles. Well water was collected from an untreated residential well located approximately 300m from the entrance to the cave. Water was stored in carboys and aerated until used. Water chemistry was analyzed by the Illinois State Water Survey and bacterial counts were conducted by Teklab, Collinsville, Illinois USA, from samples collected on 9 June 2014 (Table 1). Cave conditions were simulated by housing snails in a darkened electronic cooler set at 14^{o} C, the average temperature of water in Stemler Cave (Taylor et al. 2000). Surface (epigean) conditions were simulated by housing snails at ambient room temperature (25.5^{o} C day and 23.5^{o} C night) with 12 hours of light (108 lumens/m^2) per day.

Table 1. Comparison of nutrients and bacterial counts in well water and Stemler Cave water collected on 9 June 2014. Bacterial counts reported in colony forming units (CFU) per ml sample.

Parameter	Well water	Cave water
Nitrogen (TKN)	<0.16 mg/L	1.7 mg/L
Ammonia (NH_3)	<0.03	0.62 mg/L
Phosphorus (PO_4)	0.012 mg/L	0.224 mg/L
Fecal Coliform	<10 CFU/mL	110000 CFU/mL
Total Coliform	10 CFU/mL	280000 CFU/mL

To study growth rates, we isolated five egg masses produced by a pair of adults between 9 May and 13 May 2014 and allowed hatchlings to grow until they reached a shell size of at least 1mm. Individual snails from each clutch were placed in separate deep Petri dishes and evenly distributed across the four treatments until each treatment contained 20 snails. Snail growth rates were recorded twice a week for 7 weeks (3 June 2014 to 21 July 2014) by measuring shell length of each snail with a millimeter ruler under a dissecting microscope. Mean age of snails on the first day of data collection was 13.4 days post hatching. Snails were fed ground algae-based fish food *ad libitum* and the water in each Petri dish was changed twice weekly. Survival during the growth study was 100% for the cave treatment with well water, 95% for surface treatment with cave water, and 90% each for cave treatment with cave water and surface treatment with well water. The first instance of reproduction in each treatment was noted. A two-way AVOVA was conducted to test the influence of water type (cave vs. well) and temperature + light (cave vs. surface conditions) on overall snail growth rates (change in shell length) using Excel.

Fecundity was quantified by counting all eggs produced by four pairs of mature (circa 7 months old) lab-raised parent snails kept in plastic cups of well water. Physids are hermaphrodites and preferential outcrossers but are capable of self-fertilization (Wethington & Dillon 1997). Egg production was recorded under surface conditions for nine days, and then snails were transferred to simulated cave conditions. Data were collected for two weeks, beginning when the first snail produced eggs (3 days after entering cave conditions). Two adults died during the two weeks in cave conditions and surviving adults were combined to maintain pairs of snails. Thus, for 6 of the 14 days in cave conditions there were only 3 pairs of snails. Data are reported as mean number of eggs produced per snail per day. Embryological development rates and hatchling success rate were determined by isolating each egg mass produced by the experimental snails and monitoring embryos daily.

RESULTS

Growth rates. Data on growth rates of snails in all four treatments are presented in Figure 2. There was no significant interaction between water type and temperature + light ($p = 0.082$). Thus, the growth effect of the water type on snails is not dependent upon the temperature + light, and the effect of temperature + light is not dependent upon the type of water. Water type alone did have a significant effect on growth ($p = 0.001$). Nutrient rich cave water resulted in faster growth rates in both simulated cave and simulated surface conditions.

Temperature + light also had a significant effect on growth ($p = 0.037$). Snails reared under surface conditions had faster initial growth rates, however cave treatment snails reached a slightly larger size. Growth rates slowed at a point just prior to the onset of egg production in all treatments (Figure 2). The first snails to reproduce were reared under surface conditions in cave water and were 4.5 weeks old when the first eggs were deposited. These surface snails matured 2 weeks earlier than snails in well water and 3 weeks earlier than any snail in either cave treatment.

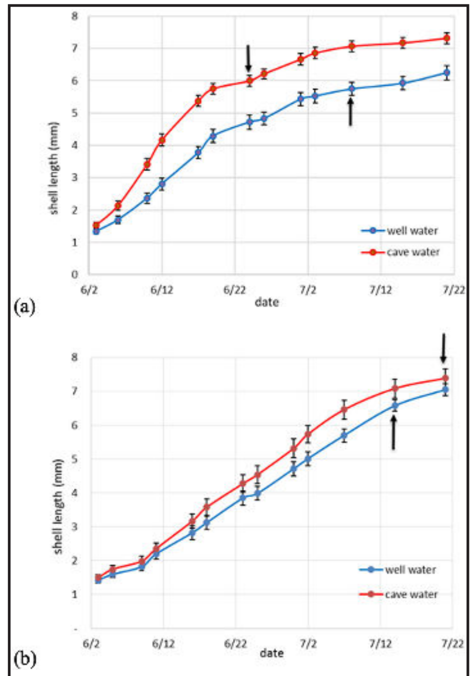

Figure 2. Mean shell size of Stemler Cave (St. Clair County, Illinois, USA) *P. acuta* raised under simulated surface conditions (n=20/water type) (a) and simulated cave conditions (n=20/water type) (b). Black arrows represent first instance of egg production. Error bars represent +/- one standard deviation.

Fecundity and embryonic development. Parent snails in the fecundity study produced 1060 eggs under surface conditions, 4.6 times as many eggs/day than in simulated cave conditions (Figure 3). Embryos under surface conditions developed more rapidly and hatched sooner than those left to develop in cave conditions. The mean development times were 11.7 days for surface and 18.5 days for cave conditions (Figure 4). Hatching success rates were higher for cave treatment embry-

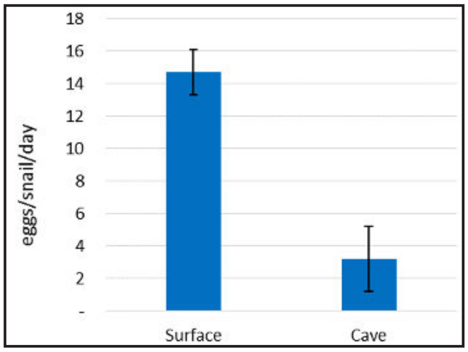

Figure 3. Mean number of Stemler Cave (St. Clair County, Illinois, USA) *P. acuta* eggs produced per snail per day under surface conditions (14.7 eggs) and cave conditions (3.2 eggs). Error bars represent +/- one standard deviation.

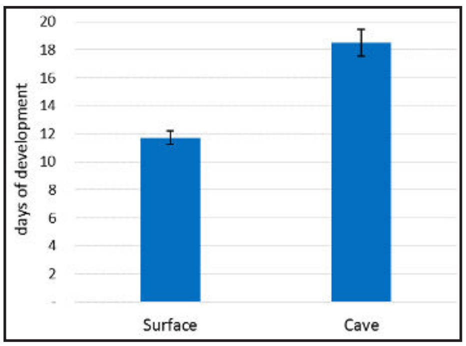

Figure 4. Mean embryonic development time for Stemler Cave (St. Clair County, Illinois, USA) *P. acuta* snails under simulated surface and cave conditions, measured in days from oviposition to hatching. Error bars represent +/- one standard deviation.

os (90.7%) than surface embryos (75%), due in part to the failure of 5 large surface clutches (n = 125 eggs) to develop.

DISCUSSION

Our study demonstrates that *Physa acuta* exhibits phenotypic plasticity in response to temperature + light and nutrient levels. Slower growth rates, delayed onset of reproduction, lower fecundity and longer development times were observed in simulated cave conditions compared to surface conditions. Weck and Taylor (2016) reported a similar laboratory study of growth rates in another taxon of cave-dwelling *Physa* snails from Fogelpole Cave in southwestern Illinois where simulated cave treatment animals had lower growth rates and achieved a larger body size than surface treatment animals. These patterns follow the temperature-size rule; growth is slower and individuals mature at a larger body size in colder environments (Atkinson, 1996). Arendt (2015) demonstrated the temperature-size rule is adaptive for *P. acuta*, as an increase in reproductive allometry compensates for slower growth. The effect of nutrient levels in the water is seen in the differences in growth rates between cave water and well water treatments. Stemler Cave water contained at least 10 times more total nitrogen than well water, 20 times more ammonia and phosphorus, and high levels of coliform bacteria (Table 1). We observed that cave water allowed biofilms to form on the bottom of Petri dishes, especially in the presence of light, providing additional food and nutrients for snails. Rollo and Hawryluk (1988) observed that growth rates and fecundity correlated with quality of diet in the snail *Physa gyrina*.

In this study, *Physa acuta* raised in simulated cave conditions in cave water reached 7 mm shell length and began reproducing at 8.5 weeks of age, compared to surface condition snails in cave water that first reproduced at 6 mm shell length and 4.5 weeks of age. The lower fecundity of cave treatment snails in our study is likely due to slower metabolic rates experienced in colder cave-like conditions.

It has been demonstrated that isolated *P. acuta* snails left to self-fertilize begin to lay eggs at a much later date than snails that are paired (Wethington and Dillon, 1993). The first instance of oviposition in all treatments of our growth study were earlier than the laboratory study of selfing in *P. acuta* reported by Wethington and Dillon (1993) and are more similar to studies of pair snails (Table 2).

Table 2. Date of first oviposition in *Physa acuta* raised in isolation and as pairs.

Source	Paired or Self	Weeks to first oviposition
Wethington and Dillon 1993	SELF	14
Dillon et al. 2004	PAIRED	6
Dillon et al. 2005 AZ pop	PAIRED	4
Dillon et al. 2005 SC pop	PAIRED	5
Current study:		
Surface conditions, well water	SELF	6.5
Surface conditions, cave water	SELF	4.5
Cave conditions, well water	SELF	7.5
Cave conditions, cave water	SELF	8.5

Although cave conditions reduced fecundity and extended embryonic development times in *P. acuta*, it appears that this species still has life history traits that could provide a competitive advantage over Fontigens antroecetes within Stemler Cave. *Fontigens antroecetes* reared in simulated cave conditions took 32 weeks to reach the minimum adult size of 2.5 mm, took at least one year to begin reproducing, had considerably slower embryonic development times (70.7 days) and much lower fecundity with one pair of snails producing only 40 eggs over 181days (Weck, 2022).

Observations from Stemler Cave. No physid snails were recorded in Stemler Cave during fieldwork by Hubricht (1941) or Peck and Lewis (1978). Since the snail is relatively common in the cave today, it is likely that *Physa acuta* invaded Stemler Cave from epigean habitats sometime after 1978. Eutrophication of the cave stream caused by septic contamination has been cited as a principle factor in the disappearance of the Illinois Cave Amphipod, *Gammarus acherondytes* Hubricht & Mackin, 1940 from Stemler Cave (Panno et al., 2006). Nutrient enrichment may also have allowed *Physa acuta* to establish a viable population in the cave by increasing formation of biofilms that snails feed on. One of us (RGW) conducted cavesnail census work between September 2009 and July 2011 that showed densities of *P. acuta* were lower than the state endangered Fontigens antroecetes in Stemler Cave (Taylor et al., 2013). However, in some subsequent surveys *P. acuta* densities were greater than *F. antroecetes*, suggesting there are fluctuations in the densities of both species.

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