Invertebrate Biology

Invertebrate Biology x(x): 1–10.
Published 2016. This article is a U.S. Government work and is in the public domain in the USA.
DOI: 10.1111/ivb.12119

Hatching plasticity in the tropical gastropod *Nerita scabricosta*

Rachel Collin,[a] Karah Erin Roof, and Abby Spangler

Smithsonian Tropical Research Institute, 0843-03092 Balboa, Panama

Abstract. Hatching plasticity has been documented in diverse terrestrial and freshwater taxa, but in few marine invertebrates. Anecdotal observations over the last 80 years have suggested that intertidal neritid snails may produce encapsulated embryos able to significantly delay hatching. The cause for delays and the cues that trigger hatching are unknown, but temperature, salinity, and wave action have been suggested to play a role. We followed individual egg capsules of *Nerita scabricosta* in 16 tide pools to document the variation in natural time to hatching and to determine if large delays in hatching occur in the field. Hatching occurred after about 30 d and varied significantly among tide pools in the field. Average time to hatching in each pool was not correlated with presence of potential predators, temperature, salinity, or pool size. We also compared hatching time between egg capsules in the field to those kept in the laboratory at a constant temperature in motionless water, and to those kept in the laboratory with sudden daily water motion and temperature changes. There was no significant difference in the hatching rate between the two laboratory treatments, but capsules took, on average, twice as long to hatch in the laboratory as in the field. Observations of developing embryos showed that embryos in the field develop slowly and continuously until hatching, but embryos in the laboratory reach the hatching stage during the first month of development and remain in stasis after that. Instances of hatching plasticity in benthic marine invertebrates, like the one in *N. scabricosta*, could greatly enhance our ability to investigate the costs and benefits of benthic versus planktonic development, a long-standing area of interest for invertebrate larval biologists.

Additional key words: Neritimorpha, phenotypic plasticity, bet-hedging, rocky intertidal, life history evolution

Hatching—escape or release from the eggshell, jelly layers, egg capsule, or brood chamber—happens at some point during the development of most animals. This transition is often viewed as a fixed point in an organism's ontogenetic trajectory and to be more or less invariant with respect to developmental stage. However, evidence is accumulating that within a species, population, or clutch hatching may vary with respect to rate, timing, or developmental sequence (reviewed in Warkentin 2011a). Such plasticity in hatching occurs in many phyla, is often environmentally induced by biotic or abiotic cues, and is often adaptive (Warkentin 2011a). Hatching, like metamorphosis, is another important life history transition, and can be induced or delayed by external stimuli once embryos have reached a stage where they are competent to respond. The duration of the delay or the period by which hatching can be accelerated is usually a fraction of the total duration of normal development, but occasionally the delay in hatching can equal or exceed the duration of development (e.g., Duguid & Page 2011; Martin et al. 2011; Armstrong et al. 2013; Branscomb et al. 2014).

Well-documented biotic cues for hatching plasticity include the effects of predators on amphibian embryos and the effects of potential hosts on parasite embryos. Adaptive plasticity in hatching time and hatching stage in response to predators was first documented in amphibians and appears to be widespread in frogs (Capellán & Nicieza 2007; Gomez-Mestre et al. 2008; Warkentin 2011a and references therein). Threats to eggs often trigger hatching and subsequent escape of tadpoles from egg predators (e.g., Johnson et al. 2003; Touchon et al. 2006; Warkentin 2011b), while threats to larvae or juveniles can delay hatching, retaining the embryo in the

[a]Author for correspondence.
E-mail: collinr@si.edu

egg until the hatchling is larger and possibly more predator resistant (Sih & Moore 1993; Moore et al. 1996). Predator induced hatching plasticity has been reported for few marine organisms, but the presence of predators delays hatching in one species of marine gastropod (Miner et al. 2010) and physical damage of egg masses similar to that caused by predators can induce rapid hatching in nudibranchs (Strathmann et al. 2010; Oyarzun & Strathmann 2011) and barnacles (Branscomb et al. 2014). In monogenean flatworms and some nematodes hatching is likewise triggered by biotic interactions, but in these cases it is the presence of host organisms or cues that indicate the presence of a host that rapidly induce hatching of infectious stages (Kearn 1986; Huang & Pereira 1994; Whittington & Kearn 2011). Other biotic cues influencing hatching include the inhibition of pond snail hatching in the presence of starving or crowded conspecifics (Voronezhskaya et al. 2004), and the probable stimulation of hatching by increased food availability in barnacles (e.g., Branscomb et al. 2014).

Abiotic cues for hatching can include light, temperature, salinity, agitation, and hypoxia. Abiotic cues can also be adaptive as they trigger the release of larvae during the point in the tidal cycle or the diel cycle that maximizes larval survival. For example, in fishes which lay their eggs above the waterline in the intertidal, hatching is stimulated by exposure to water with high oxygen content and by agitation of the eggs, both of which indicate inundation by waves during high tides (Martin et al. 2011). Mosquitoes may also use inundation to cue hatching (Vitek & Livdahl 2009). In many intertidal fishes, exposure to air inhibits hatching, preventing hatching when larvae would be susceptible to desiccation (e.g., Dimichele & Taylor 1980), but the opposite has been shown in some freshwater fishes (Wedekind & Müller 2005). Likewise, low salinity, which may be harmful to free-living larvae, inhibits hatching in sea urchin embryos (Armstrong et al. 2013). Finally, a number of coral reef fishes hatch in the hours following sunset. This pattern is maintained despite differences in temperature and salinity, and is thought to reduce predation on hatchlings (Asoh & Yoshikawa 2002; Bradbury et al. 2004).

Although hatching plasticity has been observed in a wide diversity of taxa there are few reports of this phenomenon in marine invertebrates (Miner et al. 2010; Strathmann et al. 2010; Branscomb et al. 2014). Anecdotal observations associated with descriptions of the development of species of neritimorph gastropods suggests that some species of *Nerita* show considerable variation in time to hatching and may delay hatching in the laboratory. Although they are extremely abundant and often important grazers in intertidal communities (Garrity & Levings 1981; Underwood 1981, 1984; Levings & Garrity 1983), there are few published studies of nerite reproductive ecology. What studies there are suggest that variable time to hatching may be common in the family. In the only previous study to follow capsules in the field, Przeslawski (2011) observed that capsules of *Nerita melanotragus* E. A. Smith 1884 can remain in the intertidal for up to 55 d, but that embryos in the majority of capsules reach an inferred hatching stage after 35 d. Interpretation of these results is complicated by the fact that photographs could not be used to distinguish normally developing capsules from those that had either died or hatched but remained attached to the substratum (Przeslawski 2011).

Laboratory observations also suggest that nerites may have the capacity to delay hatching. Capsules of *N. melanotragus* do not hatch naturally in the laboratory, but when opened artificially they contain fully formed normal veligers from day 25 until at least day 78 (Page & Ferguson 2013). The failure of capsules containing apparently normal veliger larvae to hatch in the laboratory has also been reported for *Nerita peloronta* Linneaus 1758, *Nerita versicolor* Gmelin 1791, and *Nerita tesselata* Gmelin 1791 from Bermuda (Lebour 1945) and for *Nerita albicilla* Linneaus 1758 from New Caledonia (Risbec 1932). Hatching plasticity induced by salinity changes has been observed in capsules of the estuarine nerite, *Neritina zebra* (Bruguière 1792). These embryos hatch after 21 d at 5 ppt salinity in the laboratory, while at 15 ppt salinity hatching was delayed and induced by a reduction to 10 ppt on day 28 (Barroso & Matthews-Cascon 2009). How much longer hatching could have been delayed is not clear.

We combined laboratory and field approaches to determine if *Nerita scabricosta* Lamarck 1822, a common nerite from the intertidal of the Bay of Panama, displays hatching plasticity. We followed individual capsules in the field to answer the following questions: (1) What is the natural duration of encapsulated development? and (2) Does the duration of encapsulated development vary with physical conditions or variation in exposure to potential predators? We used laboratory experiments to answer these additional questions: (3) Is hatching delayed in the laboratory? (4) For how long can hatching be delayed? and (5) Does exposure to water agitation in combination with sudden temperature changes induce hatching?

Methods

Natural history of the study species

Nerita scabricosta is an abundant and important herbivorous species in the rocky intertidal of Central America. This species ranges from Baja, California to Ecuador (Keen 1971; Hurtado et al. 2007). On the Pacific coast of Panama near the entrance to the Panama Canal individuals can reach a shell height of 30 mm and densities can reach as many as 400 individuals/m^2 (Garrity & Levings 1981; Garrity 1984) when aggregations in crevices are included (Fig. 1A). Herds of *N. scabricosta* leave the protection of crevices in the upper intertidal at night and follow the ebbing tide, grazing the upper and mid intertidal. Their grazing can have a significant impact on barnacle recruitment as well as species composition of algal communities (Garrity & Levings 1981; Levings & Garrity 1983). The biology of the adults is shaped by the threat of predation by fishes during high tide, and by the probability of heat stress and desiccation during low tide (Levings & Garrity 1983).

Females of *N. scabricosta* deposit their egg capsules in tidal pools in the mid and high intertidal. They show a preference for small pools in crevices (Fig. 1B), and attach the egg capsules to the smooth rock of the sides of the pool up to, but not above, the air-water interface (Fig. 1B). In large shallow pools capsules are limited to the periphery (personal observation, RC, AS, & KER). Capsules are almost never found attached to small rocks or other debris. As in most other nerites, *N. scabricosta* females produce flat, blister-shaped capsules (Fig. 1A–D), with a dorsal wall embedded with calcospherites (Kano & Fukumori 2010). A very thin basal membrane separates the contents of the capsule from the substratum, and the capsules cannot be removed from the substratum without rupturing. Capsules occasionally show evidence of predation or damage by grazers in the field (Fig. 1D). This can include both round holes probably made by boring (e.g., Przeslawski 2011) or more irregular holes, where part of the lid is broken off, similar to that described by Kano & Fukumori (2010). Capsules that have been opened by these predation attempts can be clearly distinguished from those that have hatched normally. Normal hatching leaves a white ring on the substratum after the entire lid detaches (Fig. 1C,D).

Field observations

To determine time to hatching in the field we followed the fate of egg capsules deposited in 16 pools

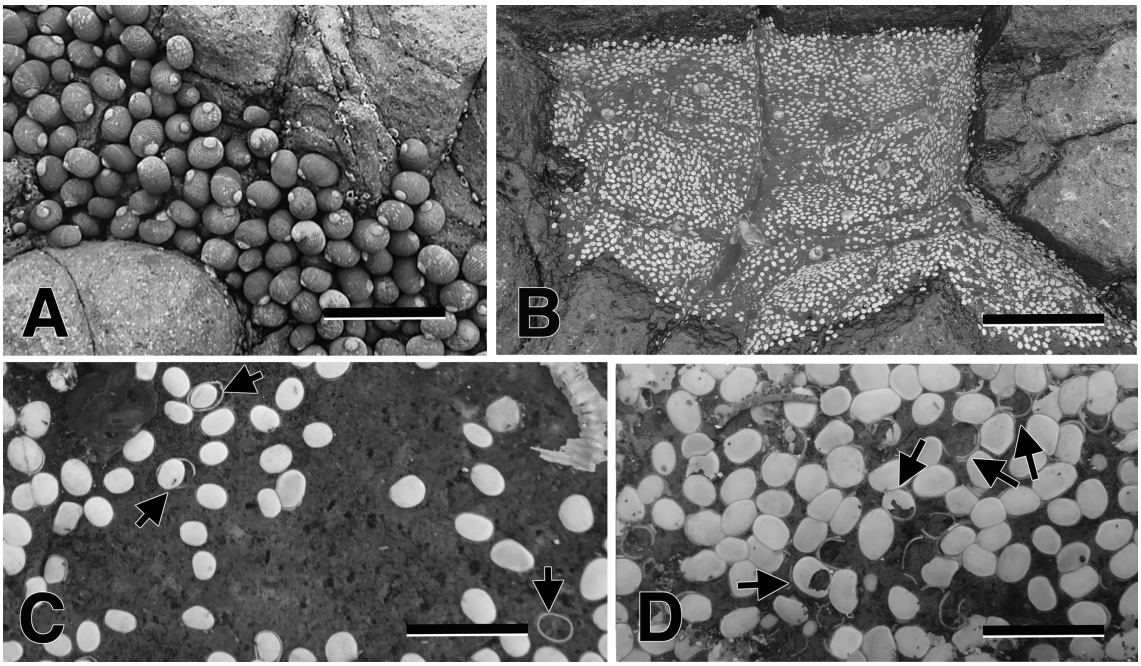

Fig. 1. *Nerita scabricosta* and their egg capsules in the rocky intertidal of Culebra Island. **A.** *Nerita scabricosta* clustered together during low tide. Scale=15 cm. **B.** A pool with *N. scabricosta* egg capsules. Scale=6 cm. **C**. Egg capsules of *N. scabricosta* showing circular marks from hatched capsules (arrows) and new capsules laid on the sites of recently hatched capsules (left arrows). Scale=10 mm. **D.** Egg capsules in a more crowded pool, showing damaged capsules (left-most arrows) and naturally hatched capsules (right arrows). Scale=9 mm.

in the intertidal of Isla Culebra (8.912027°N, −79.529682°W; Robertson et al. 2009). Quadrats in each pool were photographed starting in June 2014. Initial photographs were used to document existing capsules, and photos taken every Monday, Wednesday, and Friday from June 16–September 14 were used to document newly deposited or hatched capsules. The day that they were first observed as hatched (as indicated by the disappearance of the capsule lid leaving a clear white ring), damaged (as indicated by irregular chunks missing from the capsule top), or covered by a new capsule was reported as the last day observed.

Physical conditions in each pool were recorded during the study period. Temperature and salinity only differ between the pools during the low tide when the pools are emersed. These differences develop as the receding tide cuts the pools off from the ocean and are maximal before the incoming tide inundates the pools. As the tidal amplitude differs across the 2-week tidal amplitude cycle, the timing and duration of this exposure varies with the lunar cycle. To standardize our comparisons of the pools, we measured the temperature and salinity in each pool 3 h after the low tide on the 2–3 d with low tide around mid-day. This was designed to measure the temperature after the warmest time of the day and salinity after the greatest exposure to afternoon rains. We took measurements during five tidal amplitude cycles for a total of 14 measurements. Temperature was measured in the center of each pool using a digital thermometer. Water samples were collected at the bottom of each pool and returned to the laboratory where salinity was measured with a handheld refractometer.

Potential predators on egg capsules include chitons, crabs, and fishes (Kano & Fukumori 2010). To quantify the potential predator community in the pools, each pool was observed for 10 min during the daytime low tide, and the number of chitons, crabs, hermit crabs, and large and small fishes observed in the pool were counted. This survey was repeated eight times between June 16 and July 28, 2014.

Survival analysis was used to calculate the time to hatching for 50% of the capsules in each pool (H_{50}) and to test for a difference in time to hatching among pools. Capsules that were covered by other capsules or obviously consumed by predators were treated as censored. Analysis of variance (ANOVA) was also used to compare the time to hatching among pools (excluding censored capsules) as the two approaches are affected differently by the few capsules that took an unusually long time to hatch. Correlation coefficients were calculated to determine if mean abiotic (temperature, salinity, pool size, pool depth, and order of inundation) and biotic (potential predator abundance) factors were correlated with the H_{50} or mean time to hatching for each pool. All analyses were conducted using JMP 11.

Laboratory experiment

Capsules laid at the same time as those tracked in the field were kept in cups in the laboratory to determine if time to hatching differs between the field and the laboratory. Two treatments were applied to determine if sudden changes in temperature combined with water agitation and increased oxygenation induces hatching. Because capsules cannot be removed from the natural substratum without damaging them, we deployed pucks of marine Epoxy into the intertidal. Once covered with egg capsules, we retrieved ten pucks with eggs and assigned them to one of two treatments in the laboratory. The pucks were placed in plastic cups with 300 mL of seawater and maintained at 32°C in the dark in an incubator. For five pucks, water was changed every day using 32°C water with as little water motion as possible. For the other five pucks, we attempted to simulate the incoming tide by using 28°C water, which was agitated by rapidly sucking it in and out of a turkey baster for 1 min. Before the water change each puck was examined for hatched capsules and the water that was removed from the cup was checked for larvae to verify successful hatching. After 6 weeks, when the capsules that were observed concurrently in the field had all hatched, we reduced the frequency of water changes and capsule surveys to three times a week.

Survival analysis was used to compare time to hatching between the two laboratory treatments and the capsules that were monitored in the field. Capsules that did not hatch after 105 d in the laboratory were artificially opened and all embryos were discovered to have died and become infected with protists. These were not treated as censored and were instead excluded from the analysis because this infection had caused them to remain intact well past the dates that all the living capsules had hatched.

Observations on rate of development

To determine the time at which embryos reach the hatching stage and to understand if they delay development at a particular point in ontogeny, we examined the contents of capsules of known age

from the field and the laboratory. In November 2014, we followed the development of embryos in the field. Bright white, newly laid capsules were identified and followed, and three capsules from each of seven pools were collected each week. The contents of each capsule were gently pipetted into Ependorff tubes and observed within an hour of collection using a compound microscope. A similar approach was used in the laboratory. Starting in September 2015 four pucks with more than 30 capsules were brought into the laboratory and maintained at 28°C. Three capsules were removed from each puck (for a total of 12 capsules) and examined once every 10 d. Once the shell had developed, the shell lengths of ten embryos from each capsule were measured to determine if the embryos continue to grow as they approached hatching. On days 92 and 103, only three capsules were examined as capsules remained on only a single puck. Changes in shell length in laboratory-reared capsules were investigated using a nested ANOVA with Epoxy puck number and days in the laboratory as fixed effects and capsule as a random effect nested within puck number.

Results

Conditions in the small intertidal pools where *Nerita scabricosta* females deposit their eggs can be harsh. The average temperatures in the pools 3 h after daytime low tide ranged from 30.9°C to 35.0°C (Table 1), with the maximum reported temperature of 41.7°C in pool A0 and all but two of the pools reaching 38°C on at least one occasion. The average salinity in the pools 3 h after low tide ranged from 29.5 ppt to 35.4 ppt (Table 1). The maximum reported salinity was 43 ppt, and most of the pools reached 40 ppt on at least one occasion. On rainy days salinity could drop considerably, and we recorded salinities below 15 ppt in several pools. Potential predators varied among the pools. Over the eight daytime observations of the 15 pools, we observed a total of 78 hermit crabs, 55 crabs, 25 fish and 0 chitons. The average number of potential predators observed on each day across pools was 0–3.25 (Table 1).

Pearson correlation coefficients were low among physical characteristics of the pools. Average pool temperature was negatively correlated with minimum salinity ($r=-0.53$; $p<0.05$) and positively correlated with order of inundation during the rising tide ($r=0.86$; $p<0.0001$). Average salinity was negatively correlated with depth ($r=-0.80$; $p<0.005$) and order of inundation ($r=-0.83$; $p<0.05$), and minimum salinity was negatively correlated with order of inundation during the rising tide ($r=-0.69$; $p<0.005$, respectively). The number of observed predators increased with pool surface area ($r=0.75$; $p<0.005$).

Capsules of *N. scabricosta* take around 30 d to hatch in the field. Time to hatching varied among

Table 1. Summary of time to hatching and pool characteristics measured between June–August 2014.

Pool	Mean time to capsule loss (Days) (median, *n*, SD)[a]	Time to hatch (H_{50}) (Days) (95% CI)	Temperature (°C) mean (SD)	Salinity mean (ppt) (SD)	Pool area (m^2)	Pool depth (mm)	Order of inundation	#Observed predators/day
A0	30.2 (30, 36, 2.4)	30.5 (28.4–32.8)	35.2 (3.6)	32.6 (2.9)	0.67	98	15	0.38
B0	26.9 (28, 18, 3.8)	30.1 (27.2–33.4)	35.1 (3.9)	33.5 (7.4)	0.40	60	16	1.13
B1	30.1 (29, 16, 3.1)	33.8 (30.3–37.7)	35.0 (3.8)	32.9 (4.0)	0.64	78	14	1.75
C0	29.1 (28, 30, 5.0)	34.7 (32.0–37.5)	33.9 (3.5)	34.5 (4.9)	0.16	45	12	0
C1	28.8 (28, 32, 2.1)	29.3 (27.1–31.7)	33.8 (3.2)	34.7 (7.8)	0.15	40	11	0
D0	27.9 (25, 23, 9.7)	33.0 (30.1–36.3)	33.7 (3.0)	32.8 (3.6)	0.80	76	5	1
D1	33.1 (32, 11, 6.3)	37.8 (33.2–43.1)	32.1 (2.6)	31.8 (4.3)	0.96	120	3	3.25
E0	27.1 (28, 12, 3.2)	30.9 (27.3–35.0)	35.1 (3.4)	32.9 (4.5)	0.64	80	19	0.5
E1	24.6 (25, 15, 4.6)	25.7 (23.0–28.7)	34.7 (3.3)	30.6 (8.0)	0.36	125	18	1.63
G1	23.8 (25, 16, 2.9)	27.7 (24.9–30.7)	34.9 (3.3)	31.7 (4.8)	1.08	110	13	8
H0	31.7 (32, 23, 4.9)	31.2 (28.5–34.2)	35.0 (3.1)	29.5 (9.6)	0.37	114	20	0.75
H1	25.3 (21, 15, 11.7)	34.7 (31.0–38.8)	34.8 (3.0)	31.9 (8.2)	0.33	91	17	0.88
I0	25.1 (25, 34, 3.4)	24.0 (22.3–25.8)	30.9 (1.9)	33.9 (1.4)	0.26	40	2	0.13
I1	31.9 (23, 14, 14.1)	40.9 (36.4–45.9)	33.1 (2.8)	34.7 (2.3)	0.29	44	4	0
J1	21.9 (21, 31, 7.9)	—	33.1 (2.6)	32.8 (2.3)	0.43	142	9	0.38
K1	27.1 (28, 16, 2.8)	25.4 (22.8–28.4)	34.0 (3.5)	35.4 (2.9)	—	—	—	0

[a]Means were calculated only for capsules that were not censored (i.e., not predated or otherwise lost before natural hatching) in the survival analysis.

pools from an average of 23.8–33.1 d per pool. Analysis of variance showed that the pools differed significantly in how long it took for their capsules to hatch (df=14; SS=2166.52; F=4.50; p<0.001). A post-hoc Tukey HSD test showed that capsules in pools D1, I1, H0, and A0 took significantly longer to hatch than did I0 and G1, and the others fall in between. H_{50} values from the survival analysis including censored capsules ranged from 24 to 40 d (Table 1). Survival analysis also detected a significant effect of pool on time to hatching (χ^2=99.16; df=14; p<0.0001) and a similar ranking among pools. H_{50} for pools D1, I1, H0, and A0 were significantly larger than H_{50} for E1, G1, I0, and K1 (Table 1). Although pools varied significantly in the time to hatching, none of the physical or biotic variables we measured correlated significantly with either mean time to hatching or H_{50} for each pool (p>0.05).

In the laboratory, capsules took more than 60 d to hatch, significantly longer than the time to hatching in the field. An ANOVA comparing time to hatching for capsules in the field, the agitated laboratory treatment, and the calm laboratory treatment showed a significant difference between the capsules in the field and those in the laboratory (df=2; *N*=693; SS=214779.68; F=333.55; p<0.0001). A post-hoc Tukey HSD test showed that the overall average time to hatching in the field of 28 d was significantly shorter than 65 d to hatching in the calm treatment and 63 d to hatching in the agitated treatment. The two laboratory treatments did not differ significantly from each other. Survival analysis detected a difference in H_{50} among the three treatments (χ^2=481.79; N = 834; df=2; p<0.0001) (Fig. 2). H_{50} for the calm treatment (67.49 d; 95% CI=63.75–71.45) and the stirred treatment (62.25 d; 95% CI=59.89–64.71) differed significantly from the H_{50} in the field (30.41 d; 95% CI=29.39–31.47). Because capsules incubated in the laboratory were deposited on the pucks of Epoxy over the course of 10 d before being moved into the laboratory, the time to hatching in the laboratory should be viewed as a minimum estimate.

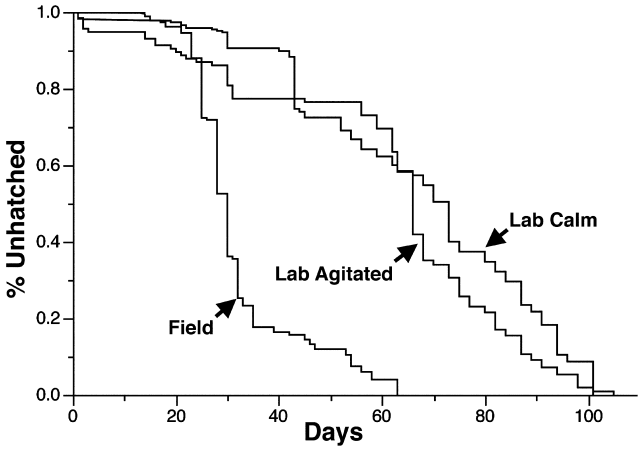

Fig. 2. Survival analysis of time to hatching for capsules deposited in June 2014 and left to develop in pools in the field compared to those brought into the lab and reared with daily agitation or in calm cultures. (*N*=692; 142 capsules censored from the field; χ^2=481.79; df=2; p<0.0001).

Observations of embryos developing in the field showed that development proceeded slowly but continuously throughout the pre-hatching period (Fig. 3). By day 21, the embryos had developed a shell length of 180 μm (SE=12.9 μm; *N*=88), which then grew to a length of 199.4 μm (SE=8.3 μm; *N*=80) by day 28. Few capsules remained in the field past day 28, but those few that were recovered on day 33 also contained larvae with shell lengths of 200.5 μm (SE=7.9 μm; *N*=41). Development proceeded similarly in the laboratory for the first 30 d. Shell length was 149.7 μm after 9 d in the lab, 184.7 μm after 20 d, and had reached 208.1 μm after 31 d (Fig. 4). An ANOVA with random effect of capsule nested within days detected a significant effect of days in the lab on shell length (p<0.0001), but no significant effect of puck. The random effect of capsule nested inside days accounted for 68% of the variation in shell length. A post hoc Tukey HSD test showed that shell length increased significantly from day 9 to day 20 and then to day 31, but that

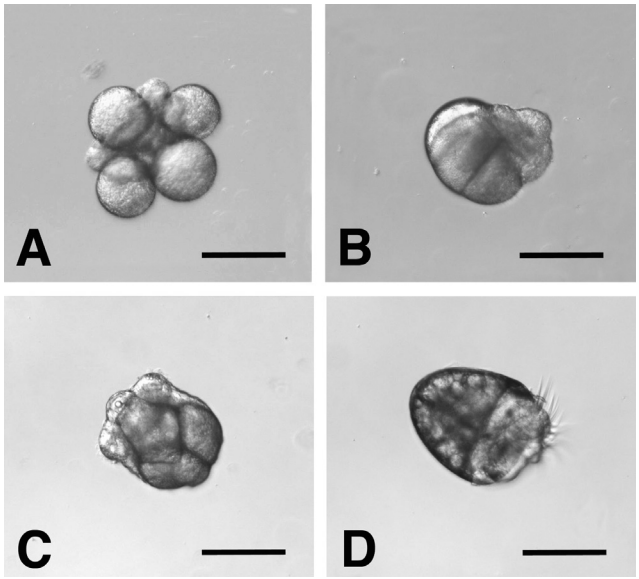

Fig. 3. Development of embryos collected from the field during November–December 2014. **A.** Early cleavage at 4–6 d. Scale=60 μm. **B.** Blastula at 8–10 d. Scale=60 μm. **C.** "Trochophore" at 13–15 d. Scale=60 μm. **D.** Hatching-stage veliger at 25–27 d. Scale=100 μm.

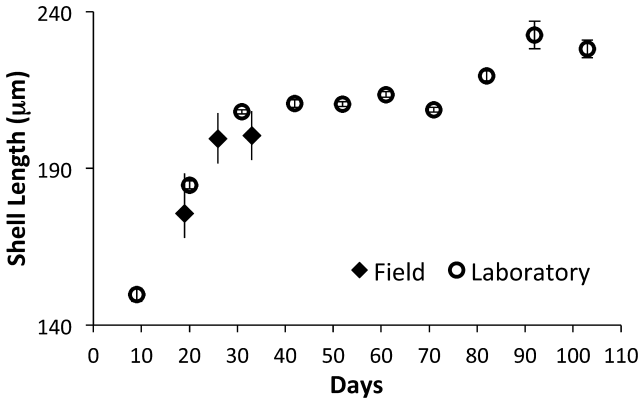

Fig. 4. Shell length of embryos removed from capsules weekly from the field (closed symbols) and every 10 d in the laboratory (open symbols). Vertical lines depict standard errors of the mean of the data and not errors of the mean for the effect from the nested ANOVA.

there were no significant differences among shell lengths measured on days 31 to 103. After day 81, embryos seemed to become more variable within any single capsule, with a number of embryos exceeding 250 µm. The small number of capsules examined and this increased variability likely account for the lack of significant difference between hatching size on days 92 and 103 and the previous days.

After being removed from the capsules, embryos often but not always took a few minutes to become active. Most of them were able to swim and appeared normal even after 2 months of incubation in the laboratory. Inverse prediction from logistic regression showed that 90% of the capsules produced swimming veligers up to 60 d in the laboratory (95% CI=43–67 d) and 50% still produced swimming veligers after 80 d (95% CI=73–92 d). Those that did not swim still showed ciliary movement in at least half of the embryos liberated from the capsules. By days 92 and 103, only 0/3 and 1/3 capsules contained swimming embryos, respectively. This supports the idea that embryos develop to a stage at which they are competent to hatch and then remain dormant until hatching is triggered, but that dormancy is not indefinite and embryos appear to lose viability after 80 d.

Discussion

Experiments with amphibians have led to recent leaps in our understanding of hatching plasticity and its impact on fitness under an array of different biotic and abiotic challenges. Unfortunately, hatching plasticity has been documented in few marine invertebrates and none have been studied in much detail. Development of tractable model systems could greatly enhance our ability to investigate the costs and benefits of benthic versus planktonic development, a long-standing question in invertebrate larval biology. Nerite gastropods may provide an ideal system for such work. Our results show conclusively that egg capsules of *Nerita scabricosta* delay hatching significantly in the laboratory compared to the field and that this delay occurs during the veliger larval stage.

The factors that stimulate or delay hatching in *N. scabricosta* have yet to be determined. Researchers who have previously observed variable hatching in nerites have suggested that agitation from waves or tidal inundation may trigger hatching (Przeslawski 2011; Page & Ferguson 2013). However, in our experiments, simulated tidal exchange (agitation and temperature change) did not alter the rate of hatching compared to still cultures. Page & Ferguson (2013) also suggested that immersion after exposure to the air could trigger hatching in *Nerita melanotragus*. However, this is unlikely to be the mechanism inducing hatching in *N. scabricosta* as the capsules are always immersed in the field (Fig. 1B). Page & Ferguson (2013) also noted that some species of nerites, but not those in the genus *Nerita*, do hatch in the laboratory. These cases all involve eclosion where the tops of the capsules detach around the edge, as they do in *N. scabricosta* (Fig. 1C,D), implying that hatching is not delayed in these cases. Our results show clearly that the capsules can and do hatch naturally in the laboratory and that, similarly to what we observe in the field, the tops of the capsules usually detach around the edge. However, hatching in the laboratory occurs only after a delay compared to capsules laid at the same time and left to develop in the field.

Temperature and salinity have been suggested to influence hatching in nerites (Barroso & Matthews-Cascon 2009; Przeslawski 2011), but capsules of the marine *N. scabricosta* held at a variety of different salinities can resist hatching for 60+ d (RC & AS, unpubl. data). Neither temperature nor salinity showed any relationship with average time to hatching in the different pools in the field, nor did a variety of other biotic and abiotic factors. These findings suggest that the observed variation among pools in time to hatching may not be a plastic response to these environmental conditions. Other environmental effects or factors like maternal effects could also influence time to hatching across pools. It is possible that the capsules we followed from each pool were deposited by one or a few females, as capsules laid on the same date could have

resulted from a single bout of egg laying from a single female in each pool. More detailed analysis of capsules deposited by individual females would be necessary to determine if maternal effects influence time to hatching in the field, or if biotic or abiotic factors other than those considered here play a role.

The pattern of hatching in the field suggests that most hatching happens tightly clustered around 30 d (indicated by a sharp drop in the line representing field capsules around 30 d in Fig. 2). After a gradual reduction in the number of capsules remaining, a second small peak in hatching occurs around 50–60 d. It is possible that this apparent second peak is due to capsules that were laid immediately over the top of hatched capsules, a pattern that we have observed to be common. Often it is possible to determine when this happens (Fig. 1), but in particularly crowded pools it may be difficult. Failures to detect these instances would effectively double the reported time to hatching. Alternately the peak in hatching around 60 d could represent a natural delay in hatching of about 10% of the capsules in the field. The 30-d time to hatching in the field is suggestive of a lunar or tidal cue; however, capsules appear to be deposited and to hatch throughout the lunar and tidal amplitude cycles (RC & AS, unpubl. data). In the laboratory, capsules do not hatch in a cluster around a certain age; rather they show a more or less continuous rate of hatching after 30 d (indicated by the steady decline in the line representing laboratory capsules in Fig. 2). This observation suggests that, in the absence of an anticipated hatching cue, capsules have a constant probability of hatching per day and are not entrained to a lunar or tidal cycle.

Developmental stasis at the pre-hatching stage is associated with extreme delays in hatching in *N. scabricosta* in the laboratory. Such extreme delays are common in freshwater fish and invertebrates (Wourms 1972; Gyllström & Hansson 2004), where they are often associated with developmental stasis or dormancy late in development. However, some groups, like certain freshwater fishes, can diapause during early and late stages of development (e.g., Wourms 1972; Furness 2015). Developmental stasis prior to hatching remains largely un-reported for marine animals, with the exception of marine copepods (e.g., Chen & Marcus 1997), a barnacle (Branscomb et al. 2014), and some fishes. Grunion, marine fishes that lay their eggs in the extreme high intertidal, are one well-known example. They can become competent to hatch in 8–10 d, but without the appropriate conditions they can delay hatching for as long as another 28 d (Martin 1999; Smyder & Martin 2002; Moravek & Martin 2011). A recent study with barnacles shows that this may also be a common strategy early in their reproductive season (Branscomb et al. 2014). Normally, brooded barnacle eggs hatch within a few days of attaining a dark color associated with mature nauplii. However, the first brood of the year can remain in this dark stage for several weeks or more than a month before hatching is triggered by an unknown environmental cue (Branscomb et al. 2014). Long diapause has also been documented early in development in the crab *Lopholithodes foraminatus* (Stimpson 1859) in which the embryos arrest for 12 months in the gastrula stage (Duguid & Page 2011). The costs of such long delays may be substantial, and it would be interesting to quantify how delays impact larval growth and survival (Hand & Podrabsky 2000; Warkentin 2011a).

Even without a delay in hatching, the development of *N. scabricosta* takes a long time compared to other gastropods developing at the same temperatures and with similarly small hatching sizes. In marine gastropods time to hatching generally correlates with the temperature at which development occurs, and the size of the embryo or hatchling. In the field, the development of *N. scabricosta* takes place between 28°C (the average seawater temperature during the wet season in the Bay of Panama) (Kerr et al. 2012; Robertson & Collin 2015) and 33°C (the average pool temperature at low tide reported here). Hatching size is around 200 μm (the shell length of mature embryos removed from the capsules). For comparison, species of calyptraeids from the Bay of Panama that hatch at 250–300 μm take only 6–10 d to develop at 28–30°C (Collin 2003, 2012), and an opisthobranch, *Tylodina fungina* Gabb 1865, with smaller larvae hatches after 13–15 d at a much colder 21–24°C (Collin 2008). A number of other gastropods from the intertidal at nearby sites take similarly short times to hatch (RC, unpubl. data), suggesting that *N. scabricosta* takes at least twice as long to hatch as is typical for intertidal gastropods in the Bay of Panama. A long time to hatching has been reported once before for a nerite. Capsules of *N. melanotragus* contain well-developed (165–180 μm) embryos after 35–55 d in the intertidal at temperatures ranging from 17–25°C (Przeslawski 2011), suggesting that slow development could be typical of nerites. What remains to be determined is why snails that already take a long time to hatch would maintain a mechanism to further delay successful hatching.

Acknowledgments. We thank Autoridad de Recursos Acuáticos de Panamá for issuing permits (No. 06, 2014; No. 75, 2015). We thank Isis Ochoa and Nerea Nieto for

help maintaining animals in the laboratory and photographing and measuring embryos, and Louise Page for sharing her translation of Risbec (1932). KER was supported by a fellowship from the National Science Foundation grant "REU 1359299: Integrative Tropical Biology at the Smithsonian Tropical Research Institute in Panama," and AS was supported by an internship from STRI.

References

Armstrong AF, Blackburn HN, & Allen JD 2013. A novel report of hatching plasticity in the phylum Echinodermata. Am. Nat. 181: 264–272.

Asoh K & Yoshikawa T 2002. The role of temperature and embryo development time in the diel timing of spawning in a coral-reef damselfish with high-frequency spawning synchrony. Environ. Biol. Fishes 64: 379–392.

Barroso CX & Matthews-Cascon H 2009. Spawning and intra-capsular development of *Neritina zebra* (Bruguière, 1792)(Mollusca: Gastropoda: Neritidae) under laboratory conditions. Invertebr. Reprod. Dev. 53: 137–143.

Bradbury IR, Campana SE, Bentzen P, & Snelgrove PV 2004. Synchronized hatch and its ecological significance in rainbow smelt *Osmerus mordax* in St. Mary's Bay, Newfoundland. Limnol. Oceanogr. 49: 2310–2315.

Branscomb ES, Vedder K, & Strathmann RR 2014. Risks for larvae mediated by plasticity in hatching. Invertebr. Biol. 133: 158–169.

Capellán E & Nicieza AG 2007. Trade-offs across life stages: does predator–induced hatching plasticity reduce anuran post-metamorphic performance? Evol. Ecol. 21: 445–458.

Chen F & Marcus NH 1997. Subitaneous, diapause, and delayed-hatching eggs of planktonic copepods from the northern Gulf of Mexico: morphology and hatching success. Mar. Biol. 127: 587–597.

Collin R 2003. World-wide patterns of development in calyptraeid gastropods. Mar. Ecol. Prog. Ser. 247: 103–122.

——— 2008. Development of *Tylodina fungina* Gabb, 1865 (Gastropoda: Notaspidea) from the Pacific coast of Panama. Veliger 50: 51–56.

——— 2012. Temperature-mediated trade-offs in the life histories of two slipper limpets (Gastropoda: Calyptraeidae) with planktotrophic development. Biol. J. Linn. Soc. 106: 763–775.

Dimichele L & Taylor MH 1980. The environmental control of hatching in *Fundulus heteroclitus*. J. Exp. Zool. 214: 181–187.

Duguid WDP & Page LR 2011. Biennial reproduction with embryonic diapause *in Lopholithodes foraminatus* (Anomura: Lithodidae) from British Columbia waters. Invertebr. Biol. 130: 68–82.

Furness AI 2015. The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. Biol. Rev. DOI: 10.1111/brv.12194

Garrity SD 1984. Some adaptations of gastropods to physical stress on a tropical rocky shore. Ecology 65: 559–574.

Garrity SD & Levings SC 1981. A predator-prey interaction between two physically and biologically constrained tropical rocky shore gastropods: direct, indirect and community effects. Ecol. Monogr. 51: 268–286.

Gomez-Mestre I, Wiens JJ, & Warkentin KM 2008. Evolution of adaptive plasticity: risk-sensitive hatching in neotropical leaf-breeding treefrogs. Ecol. Monogr. 78: 205–224.

Gyllström M & Hansson LA 2004. Dormancy in freshwater zooplankton: induction, termination and the importance of benthic-pelagic coupling. Aquat. Sci. 66: 274–295.

Hand SC & Podrabsky JE 2000. Bioenergetics of diapause and quiescence in aquatic animals. Thermochim. Acta 349: 31–42.

Huang SP & Pereira AC 1994. Influence of inoculum density, host, and low-temperature period on delayed hatch of *Meloidogyne javanica* eggs. J. Nematol. 26: 72–75.

Hurtado LA, Frey M, Gaube P, Pfeiler E, & Markow TA 2007. Geographical subdivision, demographic history and gene flow in two sympatric species of intertidal snails, *Nerita scabricosta* and *Nerita funiculata*, from the tropical eastern Pacific. Mar. Biol. 151: 1863–1873.

Johnson JB, Saenz D, Adams CK, & Conner RN 2003. The influence of predator threat on the timing of a life-history switch point: predator-induced hatching in the southern leopard frog (*Rana sphenocephala*). Can. J. Zool. 81: 1608–1613.

Kano Y & Fukumori H 2010. Predation on hardest molluscan eggs by confamilial snails (Neritidae) and its potential significance in egg-laying site selection. J. Molluscan Stud. 76: 360–366.

Kearn GC 1986. Role of chemical substances from fish hosts in hatching and host-finding in monogeneans. J. Chem. Ecol. 12: 1651–1658.

Keen AM 1971. Sea Shells of Tropical West America: Marine Mollusks From Baja California to Peru. Stanford University Press, Stanford, CA.

Kerr KA, Christy JH, Collin R, & Guichard F 2012. Reducing error in reproductive timing caused by temperature variation: Interspecific differences in behavioral adjustment by fiddler crabs. Mar. Ecol. Prog. Ser. 459: 1–16.

Lebour MV 1945. The eggs and larvae of some Prosobranchs from Bermuda. Proc. Zool. Soc. Lond. 114: 462–489.

Levings SC & Garrity SD 1983. Diel and tidal movement of two co-occurring neritid snails; differences in grazing patterns on a tropical rocky shore. J. Exp. Mar. Biol. Ecol. 67: 261–278.

Martin KL 1999. Ready and waiting: delayed hatching and extended incubation of anamniotic vertebrate terrestrial eggs. Am. Zool. 39: 279–288.

Martin K, Bailey K, Moravek C, & Carlson K 2011. Taking the plunge: California Grunion embryos emerge

rapidly with environmentally cued hatching. Integr. Comp. Biol. 51: 26–37.
Miner BG, Donovan DA, & Andrews KE 2010. Should I stay or should I go: predator- and conspecific-induced hatching in a marine snail. Oecologia 163: 69–78.
Moore RD, Newton B, & Sih A 1996. Delayed hatching as a response of streamside salamander eggs to chemical cues from predatory sunfish. Oikos 77: 331–335.
Moravek CL & Martin KL 2011. Life goes on: delayed hatching, extended incubation, and heterokairy in development of embryonic California Grunion, *Leuresthes tenuis*. Copeia 2011: 308–314.
Oyarzun FX & Strathmann RR 2011. Plasticity of hatching and the duration of planktonic development in marine invertebrates. Integr. Comp. Biol. 51: 81–90.
Page LR & Ferguson SJ 2013. The other gastropod larvae: Larval morphogenesis in a marine neritimorph. J. Morph. 274: 412–428.
Przeslawski R 2011. Notes on the egg capsule and variable embryonic development of *Nerita melanotragus* (Gastropoda: Neritidae). Molluscan Res. 31: 152–158.
Risbec J 1932. Notes sur la ponte et le développement de mollusques gastéropodes de Nouvelle-Calédonie. Bull. Soc. Zool. France 57: 358–374.
Robertson DR & Collin R 2015. Inter- and Intra-specific variation in egg size among reef fishes across the Isthmus of Panama. Front. Ecol. Evol. 2: 84.
Robertson DR, Christy JH, Collin R, Cooke RG, D'Croz L, Kaufmann KW, Heckadon Moreno S, Mate JL, O'Dea A, & Torchin M 2009. The Smithsonian Tropical Research Institute: marine research, education, and conservation in Panama. Smithson. Contrib. Mar. Sci. 38: 73–93.
Sih A & Moore RD 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. Am. Nat. 142: 947–960.
Smyder EA & Martin KLM 2002. Temperature effects on egg survival and hatching during the extended incubation period of California grunion, *Leuresthes tenuis*. Copeia 2002: 313–320.
Strathmann RR, Strathmann MF, Ruiz Jones G, & Hadfield MG 2010. Effect of plasticity in hatching on duration as a precompetent swimming larva in the nudibranch *Phestilla sibogae*. Invertebr. Biol. 129: 309–318.
Touchon JC, Gomez-Mestre I, & Warkentin KM 2006. Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. Can. J. Zool. 84: 556–563.
Underwood AJ 1981. Structure of a rocky intertidal community in New South Wales: patterns of vertical distribution and seasonal changes. J. Exp. Mar. Biol. Ecol. 51: 57–85.
——— 1984. Microalgal food and the growth of the intertidal gastropods *Nerita atramentosa* Reeve and *Bembicium nanum* (Lamarck) at four heights on a shore. J. Exp. Mar. Biol. Ecol. 79: 277–291.
Vitek CJ & Livdahl T 2009. Hatch plasticity in response to varied inundation frequency in *Aedes albopictus*. J. Med. Entomol. 46: 766–771.
Voronezhskaya EE, Khabarova MY, & Nezlin LP 2004. Apical sensory neurones mediate developmental retardation induced by conspecific environmental stimuli in freshwater pulmonate snails. Development 131: 3671–3680.
Warkentin KM 2011a. Environmentally cued hatching across taxa: embryos respond to risk and opportunity. Integr. Comp. Biol. 51: 14–25.
——— 2011b. Plasticity of hatching in amphibians: evolution, trade-offs, cues and mechanisms. Integr. Comp. Biol. 51: 111–127.
Wedekind C & Müller R 2005. Risk-induced early hatching in salmonids. Ecology 86: 2525–2529.
Whittington ID & Kearn GC 2011. Hatching strategies in monogenean (Platyhelminth) parasites that facilitate host infection. Integr. Comp. Biol. 51: 91–99.
Wourms JP 1972. The developmental biology of annual fishes. III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. J. Exp. Zool. 182: 389–414.