Neritid Gastropods Adapted to Different pH Environments: Spatial, temporal, and abiotic factors affecting life history traits of marine and freshwater snails

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Abstract. Marine and freshwater habitats of remote oceanic islands contain neritid gastropods that live in drastically different environments yet are very closely related. This study examined pH change and its effect on the physiology of both freshwater and marine gastropods of the family Neritidae. Because freshwater stream neritids have a life cycle that includes a marine stage of development, we expected that they would have a higher tolerance to pH change compared to marine neritids. To understand more about specific factors influencing distributions of different life history stages of neritid snails, new natural history information was compiled. Egg capsules were morphotyped for the freshwater neritids and two metapopulations of freshwaters neritids were found on different parts of the island. Additionally, we tested the hypothesis that embryonic viability in freshwater neritid snails would be greater than that of marine neritid snails when reared in acidic conditions. Results indicated the opposite of what we had predicted; freshwater neritids survival rate was 0% while marine neritids survival rate was 100% when reared in acidic conditions. Furthermore, we found that the calcareous egg capsule of marine neritids serves as a type of protective buffer to stressful pH conditions.

Key words: gastropods; Neritidae; environmental change; Moorea, French Polynesia; adaptation; pH; embryonic development; freshwater gastropod; marine gastropod

INTRODUCTION

Changing environmental conditions--whether natural or anthropogenic--creates challenges for organisms. Environmental stresses related to aquatic environments involve changing pH, changing temperature, amount of UV exposure, and fluctuations in salt and mineral content. These affect both freshwater and marine animals in different ways and to different degrees depending on the life stage. In addition to other types of causes of habitat degradation or environmental stress, there is mounting evidence for the deleterious effects of climate change on organisms, particularly those living in marine environments (Ellis *et al.* 2009). Animals may deal with these challenges in a variety of ways including various forms of adaptation. Understanding how animals respond to these challenges is important for predicting how they might respond to future changes and finding ways to overcome or avoid these challenges.

The island of Moorea, French Polynesia contains a family of gastropods with genera that occur in either marine or freshwater habitats. Therefore, this family has individuals that live in habitats significantly different in pH; freshwater streams tend to have a lower pH than water from the marine habitat. On remote oceanic islands stream species of neritids are thought to have evolved from marine forms (Ford and Kinzie, 1982). Moorea provides a unique opportunity to study

FIG 1. Map depicting the location of collection and study sites for neritid snails on Moorea, French Polynesia. Numbers correspond to site names and information listed in Table 1. Afareaitu represents sites 1-3 in Table 1.

animals that are so closely related but inhabit very different environments. Although tropical stream neritids inhabit freshwater environments, they do retain an element of their ancestry by having an early stage of development that requires the marine environment. A life cycle known as diadromy or amphidromy, upon hatching the larvae are swept into the ocean to further undergo and complete larval development until they are ready to migrate back upstream to complete their life in the freshwater habitat (Resh *et al.* 1992). This life strategy subjects them to very harsh conditions with a taxing migration to and from the ocean. Additionally, the necessity to tolerate rapid shifts in pH while migrating between the two environments compounds the challenges posed by this life strategy (Resh *et al.* 1992). Neritids of the marine intertidal zone are strictly marine inhabitants and also experience harsh and highly variable environments that are characteristic of the intertidal zone.

Negative effects on embryonic shell development in gastropods have been found to occur in low pH conditions but little has been studied with regard to encapsulated embryos (Ellis *et al.* 2009; Fernandes *et al.* 2012). A recent study examining the combined effects of pH and temperature on encapsulated embryos of a marine gastropod in Moorea, French Polynesia found that rearing in these environmentally stressful conditions during early developmental stages had deleterious effects the organism's physiology (Allen 2012).

Embryonic encapsulation, a character shared by members in the family Neritidae, has been thought to provide a means of protection from different environmental stresses (Przeslawski 2004). The calcareous egg capsule seems to be an important part of neritid life history (Przeslawski 2004) shared by both marine and freshwater neritids. The egg capsule is the location where the embryos develop until they reach their larval stage and are released as veliger larvae that live within the plankton. As reviewed by Przeslawski (2004), there is much to be studied regarding the role of embryonic encapsulation, whether or not these capsules act as a buffer from the environment as studies have suggested (Przeslawski *et al.* 2004), and how this affects the development of embryos during early life stages when faced with environmental stress. A few studies have reported differences in structure as well as protective properties of egg capsules laid by different species

(Przeslawski *et al.* 2004; Barrosso and Mathews 2009).

This study focused on nerite gastropods of two types: marine and freshwater. Marine and freshwater neritids have similar reproductive strategies except that the marine forms are restricted to marine waters. The ways that these neritids are affected by environmental stresses, such as pH change, may differ. On the one hand, we might expect marine neritids to have a broad tolerance to environmental stresses due to the extreme nature of their intertidal habitat, as reviewed by Przeslawski (2004). On the other hand we might think freshwater stream neritids would have a broad tolerance to these stresses due to the fact that they occur in both marine and freshwater habitats during their life because of their amphidromous nature.

The first objective of this study was to document new natural history information about the different life stages of neritids and how the abundances and distributions of these stages change with time and with habitat structure. Next, we sought to test the effects of environmental stress on the physiology of neritid gastropods adapted to different pH environments. Specifically, how does low pH affect the embryonic development of amphidromous and marine neritid snails? We tested the hypothesis that embryos of amphidromous neritid snails will have a higher hatching success than those of marine neritid snails when reared in acidic conditions.

METHODS

During the month of October, *Nerita argus* were collected from the intertidal zone at site 1 and freshwater members from the family Neritidae were collected from the streams of Afareaitu watershed, sites 2-4, about 1.7 km upstream from the mouth of the river (Fig. 1,

TABLE 1. Survey and collection sites

Collection Site:	Site Number:	GPS Coordinates:
Opunohu Bay	1	17°31'4.69"S 149°51'3.83"W
Afareaitu	2	17°32'15.16"S 149°47'50.12"W
	3	17°32'16.11"S 149°47'49.95"W
	4	17°32'15.72"S 149°47'49.68"W
Opunohu Watershed	5	17°31'14.55"S 149°50'53.16"W

Table 1). Freshwater neritids were collected from the Opunohu watershed, site 5, on November 4th. Individuals were transported back to the lab to assess fecundity, identify egg morphology and examine the influence of acidified conditions on their physiology during early life stages.

Collection and maintenance of Neritids

Adults *Nerita argus* (N=38; Appendix A) were collected from the right bank of the intertidal zone adjacent to a small stream output in the Opunohu Bay of Moorea, French Polynesia (Table 1, Site 1) (identification confirmed by specimen pictures from database of Moorea Biocode Project). Adults were collected from underneath or in close proximity to rocks that contained an abundant amount of freshly laid egg capsules (older capsules begin to darken compared to new capsules, J. Capdevielle, pers. obs.). All adult *N. argus* were collected between October 1st-16th, 2013. At the same time, rocks with an algal film were collected as a potential food source and as egg laying habitat. Snails and rocks were transported to a laboratory at the UC Gump Field Station where they were housed together in a tank (76L) with a continuous flow through system of aerated seawater from Pao Pao Bay. The snails were permitted to move freely and graze on the algae covered rocks.

Adults of *Neritina canalis* (N=3: Opunohu; N=45: Afareaitu), *Neritina turrita* (N=8: Opunohu; N=6: Afareaitu), *Septaria porcellana* (N=10: Opunohu; N=5: Afareaitu), and *Clithon spinosa* (N=6: Opunohu; N=0: Afareaitu) were collected from four sites along two different watersheds. Neritids from Afareaitu watershed were collected from two waterfall locations and one free flowing stream site, including individuals collected from both pools and riffle habitat (Table 1, Site 2, 3, 4). Neritids from Opunohu watershed were collected from site 5 located underneath a bridge about 0.4km from Opunohu bay (Table 1, Site 5). Adults were collected if found in the proximity of an abundant number of egg capsules (could not differentiate between fresh and old capsules of freshwater neritids in the field). All adults were collected between October 13th-November 4th, 2013. In the field and during transport to the lab, adults were housed in containers (1.0L-2.5L) separating individuals into species specific containers. In the lab, these containers were placed in a tank (60L) and holes were made in each container so water could flow in and out of the containers. Water was supplied from a hose and PVC pipe system that dripped water into these containers and flowed through water that came from the upper reaches of the island's freshwater streams.

Seawater acidification

Water was obtained from Cook's bay and 1200ppm of CO_2 was added to lower the pH by a magnitude of about 0.4. Five-liter jugs and 1.5 liter bottles were used to collect treated water on 22 October 2013. Bottles were stored in the coolest area available (70°C, inside the lab) and in the dark to minimize photosynthesis and maintain a low pH. pH of stored water was recorded each day. The pH was measured using a pH meter (HM Extech pH100 ExStik waterproof meter).

Freshwater acidification

Water was obtained from a hose that is supplied by water from the uppermost reaches of the streams on Moorea. HCl was added to freshwater until a pH change was obtained of the same drop in magnitude as that of the acidified marine water for the Nerita experiment (~pH change of -0.4). The pH was measured using a pH meter (HM Extech pH100 ExStik waterproof meter).

Labratory procedures for Nerita argus

Egg capsule deposition and treatments

Rocks were periodically photographed beginning October 20th to make note of all already existing egg capsules to ensure correct age of newly deposited egg capsules for experimental purposes as well as fecundity surveys. For the treatment experiments water was changed every 24 hours around 10pm and pH was recorded before and after water change to ensure the capsules were being subjected to consistent treatment conditions of pH.

Embryonic development

Rocks were only examined once each day to ensure maximum amount of egg capsules laid in one batch. Examination took place around 10AM each morning until large enough batches were laid in one night for the treatment and controlled conditions of the experiment and to ensure eggs were of the same age and assumed to be the same egg laying event therefore from the same
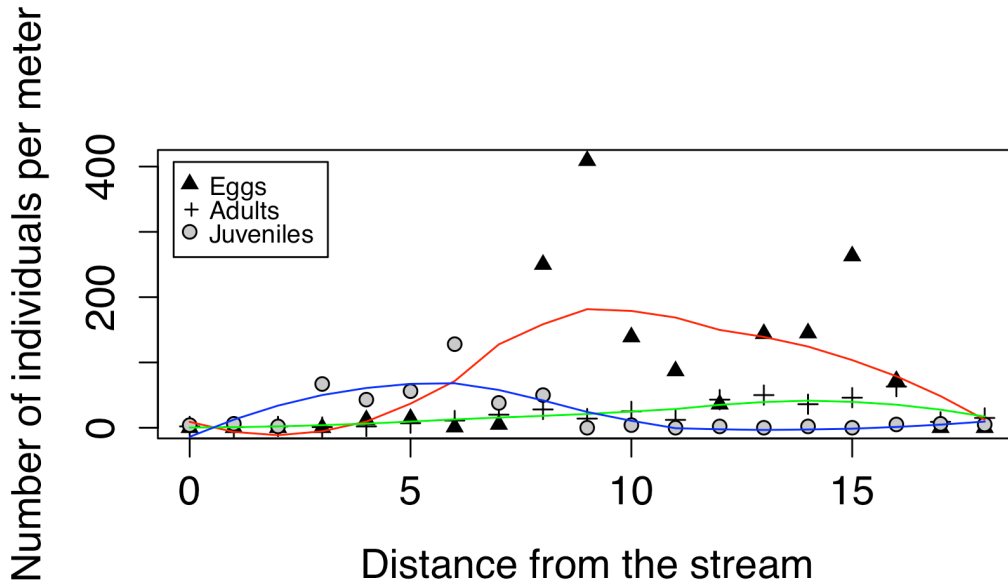
FIG. 4. The abundance of different life stages of *Nerita argus* as distance from the stream output changes.

individual. Once two initial egg batches were laid of a large enough sample size (N = 28) and on separate rocks, rocks were immediately removed from tank and placed in containers (500mL) of treated marine water (pH~7.7) and untreated marine water (control: pH~8.1). One egg capsule from the treatment (low pH) batch and from the control pH batch were then removed every other day with a razor blade. Upon removal, egg capsules were placed in individual sealed petri dishes (volume = 96mL) with their respective treatment waters that were changed each night. The transparent underside of each capsule, normally protected by the rock, allowed for observation and imaging through the dissecting scope (Leica MZ16) using a digital camera (Leica Panasonic) to qualify developmental stage and rate. This continued for a period of 21 days until the intracapsular embryos had reached full development or hatched.

Embryo viability in acidic conditions: removed vs. non-removed

Once batches for developmental experiments were obtained, rocks were then examined between 3-5 times each day to observe egg capsule deposition. Rocks containing new eggs were immediately placed in treatment waters prior to egg capsule removal from rock. Egg capsules that were removed from the rock within the first 10 days of being deposited (N = 5) were cut open with a razor blade once they reached 20 days of age. The inner contents of the capsules were examined through a dissecting scope (Leica MZ16) to assess embryo viability. A control sample of non-removed egg capsules remained attached to the rock throughout the 20 day period, kept in their respective
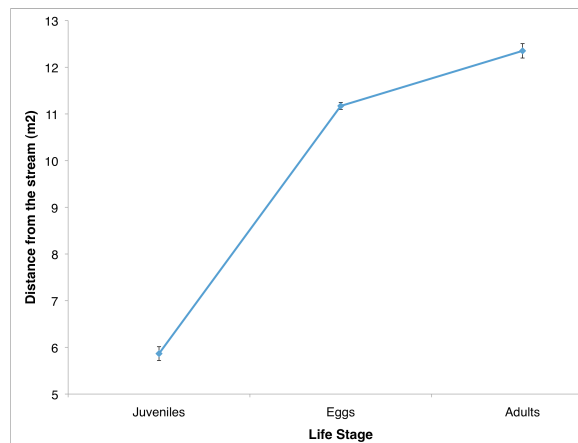

FIG. 5. Values represent the mean distance each life stage is found. Tukey's-HSD test, F=601.69, P<.0.001. Vertical bars indicate +/- one standard

treatments of low pH and control pH marine water. In order to assess embryo viability, the capsules were removed from the rock after 20 days and examined with a dissecting scope via the previously mentioned procedure.

Viability was measured as number of veligers displaying any activity divided by the total number of veligers. Two-way analyses of variance (ANOVAs) were used to investigate the effects of pH and removal on embryonic survival. All analyses were conducted in R (R Development Core Team 2013).

Fecundity and egg morphology

Rocks were examined multiple times a day and noted how many new eggs were found (see appendix D for data). Photos were taken to create a guide to key out egg capsule types (appendix B).

Laboratory procedures for Neritina spp.

Egg capsule deposition and treatment
Rocks were photographed beginning October 20th to make note of all already existing egg capsules. Rocks were examined throughout each day to observe egg capsule deposition. Once two egg batches were laid of a large enough sample size (N=6) by *Neritina turrita* and batches were laid on separate rocks, rocks were immediately removed from tank and placed in containers (500mL) of treated freshwater (pH=7.0) and untreated freshwater (control: pH=7.40). Water was changed every 24 hours at 10pm and pH was recorded before and after water change to ensure the capsules were subjected to consistent treatment

conditions of pH.*

Embryonic viability in acidic conditions: freshwater taxon vs. marine taxon

Rocks with attached *N. turrita* egg capsules were removed from treated waters at the last day of the experiment—15 days from egg deposition—following the same procedure as above for the non-removed egg capsules of *Nerita argus*. As explained previously, the *N. turrita* capsules were to be compared to those of *N. argus* capsules that remained attached to the rock throughout treatment.

Capsules were opened with a razor blade while examining inner contents through a dissecting scope (Leica MZ16) to assess embryonic viability. Viability was measured as percent of viable embryos by slicing open each egg capsule and taking a random subsample of embryos through a pipette and counting the total number of embryos that appeared to be alive, in an early developmental stage (not yet to eyespot formation) and dividing that by the total number of embryos within that pipette subsample. These numbers were then averaged to obtain the mean percent viability from the treated capsules reared in low pH freshwater and the untreated capsules reared in regular pH freshwater. Two-way analyses

*pH of freshwater increased to nearly 8.0 by the time of the next water change while marine pH increased by only 0.1 by the time of the next water change.
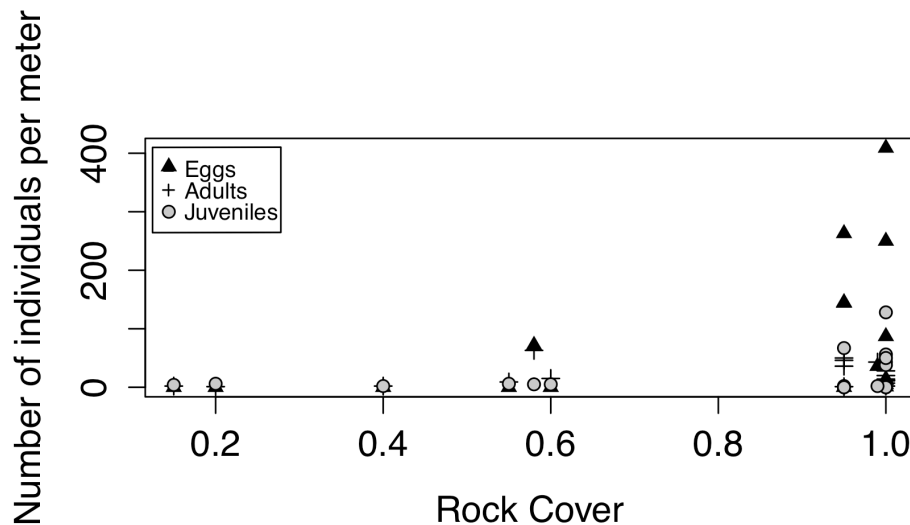

FIG. 6. The abundance of different life stages of *Nerita argus* as proportion of rock cover changes.

of variance (ANOVAs) were used to investigate the effects of pH and taxon (*N. argus* or *N. turrita*) on embryo survival. All analyses were conducted in R (R Development Core Team 2013).

Fecundity and egg morphology

Rocks were examined multiple times a day and noted how many new eggs were found (see appendix D for data). Photos were taken to create a guide to key out egg capsule types (appendix B).

Field survey

Microdistribution of life stages of Nerita argus

On October 18th, the abundance and distribution of adults, juveniles and eggs belonging to *Nerita argus* (species confirmed by photos from Moorea biocode database and Professor Vince Resh) was surveyed using a belt transect of 18m along the intertidal zone of Opunohu Bay. The transect began at the mouth of the stream and ran along the intertidal zone from the right bank of the Opunohu stream output located in front of the CRIOBE research station (Table 1, Site 5). Percent rock cover was assessed every meter using a quadrat of $1m^2$. Abundance of each life stage was plotted against distance and rock cover. One-way analysis of variance (ANOVA), using the general linear model, was used to determine the effect of life stage, distance and rock cover on the abundance of each life stage. All analyses were conducted in R (R Development Core Team 2013).

Patterns of juvenile density

In order to observe any fluctuation or change in the density of *N. argus* juveniles at different time points, site 4 was visited between October 14th and Nov021r 20th on four separate occasions, each roughly one week apart. Between one and three of the highest density rocks that were spotted were photographed and measured. Rocks were imaged and later analyzed on a computer to determine the number of juveniles on each rock. The surface area of each rock was estimated using imageJ (Rasband 1997-2012). The density of juveniles was calculated by dividing the number of individuals by the total surface area of the rocks where they were found.

Fecundity in situ: Afareaitu vs. Opunohu

Beginning on October 13th (site 1) and November 5th (site 5) fecundity was measured in two metapopulations of Neritidae over the course of 10 days. Settlement plates and tiles were placed in the stream at each site and two large rocks were scraped clear of all eggs, photographed, and revisited to determine if egg laying was occurring within any freshwater neritid species.

Statistical methods

A two-way ANOVA was used to test effects of pH and removal on embryonic survival of *Nerita argus.* A two-way ANOVA was used to test effects of pH and taxon (freshwater neritid or marine neritid) on embryonic survival. A general additive model (GAM) was used to fit curves to the graph showing abundance of life stages against distance from the stream while a general linear model (GLM) was used to look at the effects and interaction of life history stages, distance, and rock cover. A one-way analyses of variance (ANOVA), followed by a Tukey's-HSD test was used to test differences in mean distances each stage was found as well as to test differences in mean proportion of rock cover each stage was found.

RESULTS

Laboratory results

Description of egg capsules

In captivity, *Nerita argus, Neritina turrita,* and *Septaria porcellana* deposited egg capsules on basalt rocks. *Neritina canalis*, *Neritina auriculata,* and *Clithon spinosa* did not lay eggs in captivity but capsules were observed in the field and brought back to the lab for examination. Identification was confirmed by Professor Vince Resh. Egg capsules of each species differed morphologically (Appendix B).
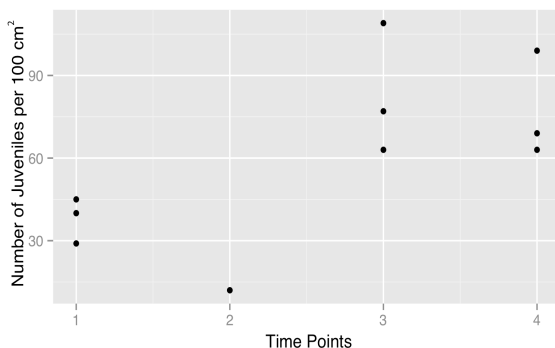

FIG. 7. The density of *Nerita argus* juveniles during four different visits to the intertidal zone at site 4. Points represent rock samples measured.

N. argus: capsules were laid individually with numbers ranging from 1-28 capsules in captivity, averaging anywhere from 1-5 mm apart. Capsules start off a bright white color and gradually darken to an off white color as embryos develop. Capsules are deposited over indentations in the rocks with a concave cap covering for the capsule wall that is visible. Capsules vary in their circumferential shape depending on the shape and size of the indentation they are housed in.

N. turrita: capsules are laid individually and underneath the microscope are nearly identical to *Neritina canalis* with a slight resemblance to the marine *Nerita argus* in that they are first deposited with a bright white coloration and seem as though they are laid in batches, but individually spaced apart. As opposed to *N. argus,* a hole or indentation does not appear to be necessary to house the capsule. Capsule has a slightly domed cap and a relatively thick capsule wall compared to *N. argus*. Capsules are consistently oval in shape.

S. porcellana: capsules are laid in clusters ranging from 3-40 and are laid in clusters with 0.5 mm separating each capsule. Each capsule is smooth with a slick outer capsule lid. Capsules start off with a pale peach coloration and with time darken and become salmon pink, in color. They have more of a circular shape in some batches while others are slightly more oval.

Neritina canalis: capsules are laid in clusters ranging from 2-100 or more and are grouped very close, nearly all of the capsules touching the adjacent capsule. Each capsule appears to be rough with sand-like particles built into the capsule wall and is off white in color to begin with while gradually darkening with time.

Neritina auriculata: capsules are very similar to *Neritina turrita* but were found to be smaller in size, which could be a result of the size of the snails, not necessarily an identifying character. They were spaced apart in a similar manner as *N. turrita* (photos unavailable--nearly identical to *Neritina turrita,* see Appendix B, Fig. 3, right)

Clithon spinosa: egg identification unknown.

Response to treatment

Developmental rate was not found to be different between the two treatments. Upon removal from the rock and examination of stage, there seemed to be much variation within each of the treatments. On average, the first sign of movement was observed at 6.7 days for control and 8.3 days for the embryos reared in acidic conditions. The first sign of eyespot formation was observed on average at 12.5 days for both treatments. Only two of the capsules that had been removed from the rock and placed in petri dishes had hatched on their own at 21 days. Other capsules were artificially opened but veliger larvae appeared to be fully developed.

The proportion of embryos of the marine neritid, *Nerita argus*, that survived was significantly dependent on the interaction of being attached to the rock and the pH of the water (P<0.001, Fig. 2). There was a much lower survival rate in the embryos that were
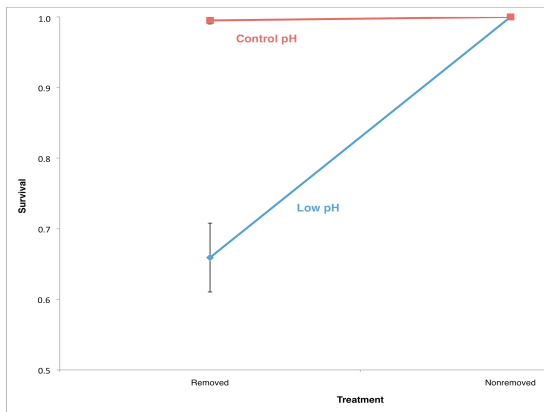

FIG. 1. *Nerita argus*. The survival of embryos incubated in control and reduced pH seawater at both removed and non-removed states. Values represent the mean percent of embryos that survived. Vertical bars indicate +/- one standard error.
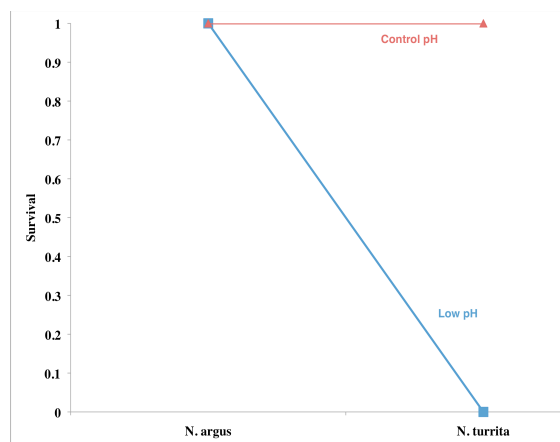

FIG. 2. *Nerita argus* and *Neritina turrita*. The survival of embryos from N. argus and N. turrita incubated in both control and reduced pH seawater treatments. Values represent the mean percent of embryos that survived. Error bars were constructed using standard error

in OA and removed from the rock during the 1st half of their development. The effects of pH depends on whether or not the eggs are removed from the rock.

There was significant interaction between taxon and pH (P<0.001, Fig. 3).The proportion of embryos of the freshwater neritid, *Neritina turrita*, that survived was dependent on the pH of the water. There was no survival in the embryos of *N. turrita* that were in acidified conditions while there was 100 percent survival in embryos of marine *N. argus*. The effects of pH depends on the taxon.

Field Survey Results

Microdistribution of life stages of Nerita argus

The abundance of each life stage of *Nerita argus* significantly depended on the distance away from the stream as well as the stage of the snail present (glm(quasipoisson), $F_{2.47}$=11.42, P< 0.005, Fig 4, Table 2). The number of juveniles was highest at 3-8 meters away from the stream output, while abundance of eggs and adults were much lower at this distance around 2-3 meters. At 7 meters, abundance of adults and eggs began to increase while juvenile abundance began to decrease. At 8-16 meters from the stream the relatively high abundance of eggs and adults is paired with an abundance of juveniles that is much lower than in areas closer to the mouth of the stream. The mean distances that eggs, juveniles, and adults were found were significantly different from each other (F=601.69, P=0)

The abundance of each life stage of *Nerita argus* significantly depended on the proportion of rock cover (glm(quasipoisson) P< 0.005, Fig. 6, Table 2). The highest abundance for all of the life stages was found where there was 100 percent rock cover. Abundance of all life stages significantly declined as rock cover decreased. There were no eggs, juveniles, or adult snails present where rock cover was less than 50 percent. The average proportions of rock over where juveniles adults and eggs were found were not

TABLE 2. Response variable: Abundance of life history stage.

Source	Chisq	Df	Pr(>Chisq)
Life stage	17.407	2	0.000166
Distance	4.4903	1	0.034087
Rock prop.	23.7653	1	1.09E-06
stage:distance	13.6556	2	0.001083

significantly different (F=1.46, P<0.24).

Patterns of juvenile density

Density of juveniles fluctuated with time during different visitation periods (Fig. 7). There was no pattern in abundance of increasing, decreasing, or remaining constant. The second time point showed a marked drop in density. The measurement of density during the third time period was almost an order of magnitude higher than the second time period.

DISCUSSION

Response to treatment

Contrary to studies examining the effects of low pH conditions on embryonic developmental rates (Ellis *et al.* 2009; Allen 2012), no obvious differences were observed in the developmental rates of treated and untreated egg capsules of *Nerita argus*. This suggests that pH may not be affecting embryos of *N. argus* while attached to the rock due to their capsular protection and the results of the pH and removal experiments. Something notable that we observed was that capsules removed from the rock to further develop in petri dishes for a few days were further along in their development than those that developed solely on the rock. This suggests that perhaps the underside of the capsule might be shielded from oxygen and-- without the rock protection--the removed capsule undergoes more rapid development due to increased availability of oxygen.

The low rate of survival in the embryos reared in acidic conditions without the protection of the rock, combined with the high rate of survival in acidic conditions while attached to the rock suggests the calcareous capsule covering serves as some kind of buffer to the effects of pH. These results are consistent with findings from past studies that have found protective properties to exist in either the intracapsular fluid or the egg capsule itself (Przeslawski 2004). The capsules that were removed from the rock in controlled conditions had high rates of survival suggesting that the low rate of survival in the removed, acidified treatments was influenced by pH.

The inability of the freshwater embryos to tolerate low pH while attached to the rock suggests they are not equipped to deal with as drastic changes in pH as their marine relatives. Perhaps tolerance to pH change is an

ancestral trait that was lost in freshwater descendents whose freshwater streams are of a relatively stable pH, which may possibly be due to the basalt bedrock of these volcanic island streams, as Professor Vince Resh has suggested. The freshwater neritid habitat maintains a stable pH as opposed to their marine ancestors whose intertidal habitat experiences rapid fluctuations in pH. Because of these fluctuations, the marine neritid might have been strongly selected for to have a high pH tolerance built into its capsular envelope.

Field survey

The fluctuation in the density of *Nerita argus* juveniles on rocks at the intertidal zone suggests that juveniles are moving and are the same individuals being examined through time or that they are declining for various reasons followed by recruitment spike. They could be the same individuals between time points 1 and 3 and the low observance of density may be a result of movement. If they are not the same individuals then that might suggest that they have a high mortality due to predation, or harsh environmental conditions and the fluctuation through time is a result of mortality followed by recruitment spikes.

The distribution of the different life history stages of *Nerita argus* at the intertidal zone in conjunction with rock cover emphasizes not only the necessity of rocks as a medium in which they deposit eggs and reproduce, but also the need for protection and anchorage from being swept out to sea. Perhaps the juveniles, located closer to the stream, are moving towards a source that provides resources that are more important to their particular life stage. It might, in fact, not have anything to do with the stream location and perhaps it is coincidence that they happened to occur at the stream mouth as they were primarily aiming to migrate to an open location with more available resources. Future surveying of the distribution of juveniles, adults, and eggs at multiple sites along the intertidal zone would be interesting to see if there is any pattern in partitioning of habitat or if perhaps juveniles are typically found closer to freshwater stream outputs.

The occurrence of the freshwater neritid populations with different characteristics in different watersheds suggests these different morphological and phenological traits might be a result of their differing habitat conditions. Perhaps individuals are selected for that have a cycle that is in accordance with the abiotic

factors of that particular watershed. For example, the location of the neritid population in Opunohu (site 5) is nearby a site that has been previously cited to be an area subjected to agricultural runoff (Liu & Resh 1997; Resh *et al.* 1990).

CONCLUSION

This study demonstrates just some of many factors, biotic and abiotic, influencing distribution, abundance, and life history traits of neritid snails. The potential for organisms to adapt or acclimate to environmental change is important to understand for future predictions of how organisms might respond to future environmental changes. The evolutionary history of these organisms raises the question as to whether or not they would be able to transition back to the marine environment, particularly, with regard to animals like *Neritina auriculata,* a freshwater neritid who has been found to inhabit both fresh and marine environments equally well (Liu and Resh 1997).

The distributions of freshwater neritid snails on the island of Moorea, French Polynesia are influenced by many interacting biotic and abiotic factors such as migrational patterns of juveniles (Frost & Schneider 1986), water quality, hydrodynamics and habitat structure (Dugan 2010). Results from this study shed light on the fact that watersheds as a whole may have habitat characters that differ from one another and act in a way that metapopulations are created, unique to a particular habitat. Future research comparing neritid populations in each watershed on the island might elucidate whether it is the natural structure of the environment or if it might be something different, for example human mediated disturbances such as agricultural practices that are creating these differences between watersheds.

It is shown here that these organisms have adapted to their different pH environments in unique ways and do not respond similarly to the same environmental changes. Although my hypothesis was not supported, the results were interesting and in retrospect are consistent with the natural history of these snails and what their specific habitats might require. These results are consistent with what we might expect from the relatively constant environments of volcanic streams, particularly as this site as has been confirmed by Resh *et al.* (1990) when characterizing this site to that described by Maciolek & Ford (1987). There

must have been selection for an animal with a broad pH tolerance due to the varying nature of this habitat. The transition into a more stable pH environment like the freshwater streams of Moorea may have occurred with a loss of this trait of pH tolerance because it was no longer necessary. It would be interesting to further investigate the threshold pH at which freshwater neritids can survive above and below their normal pH habitat levels and make inferences as to whether a shift back to the marine habitat might be possible.

Results from this study demonstrate how animals might be affected during different life stages by changing environmental factors: adults are possibly living in an environment full of agricultural runoff which might induce fertility because of a stressful environment, creating a population of neritid snails with a different phenology than populations in other watersheds. Furthermore, the affect of pH has been found to be detrimental to the physiology of animals especially during early life history stages as has been shown in other marine gastropods (Allen 2012; Ellis *et al.* 2009). Here, our results show how animals adapted to very different habitats respond quite differently to the same environmental change where one might have evolved the means to protect against this stressor but with that protection removed we see that its embryonic viability are still affected to some degree.

As we can see, animals may deal with these challenges in a variety of ways including various mechanisms of adaptation. Although time might allow organisms to respond appropriately, that is not always the case and these habitat changes might call for human mediation. While environmental change is a naturally occurring phenomena it can also be significantly influenced by human practices. It is important to understand how organisms respond to not only predict how they might respond to later environmental changes and how we might be able to help, but also to better understand the impact we have on these organisms and what we can do to reduce this.
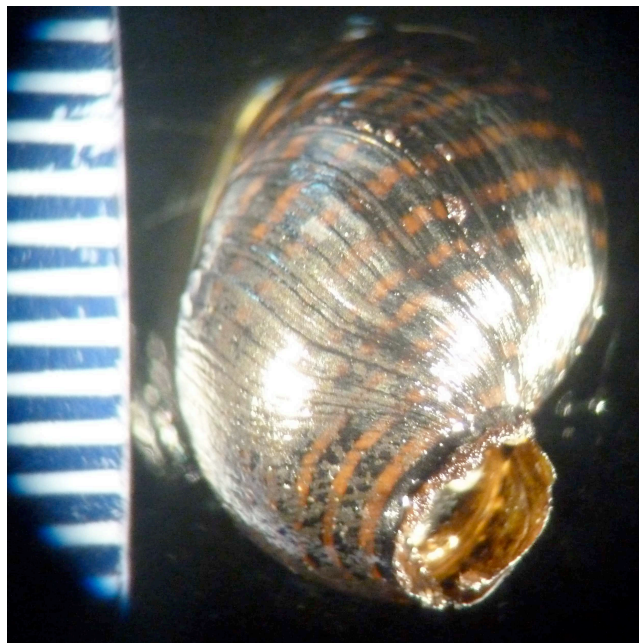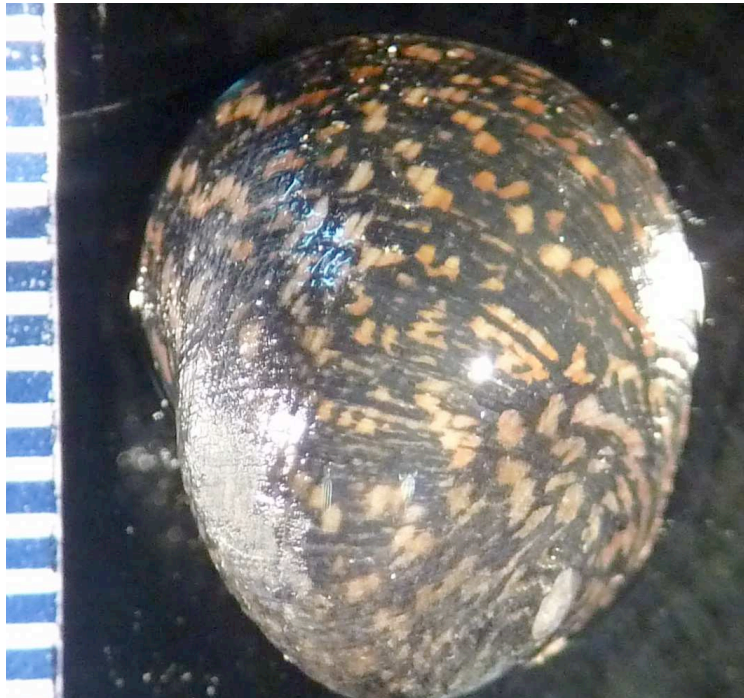
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APPENDIX A

Adult, *Nerita argus*, marine neritid snail collected on October 18th 2013. Scale bar ticks to the left of specimen (mm.)

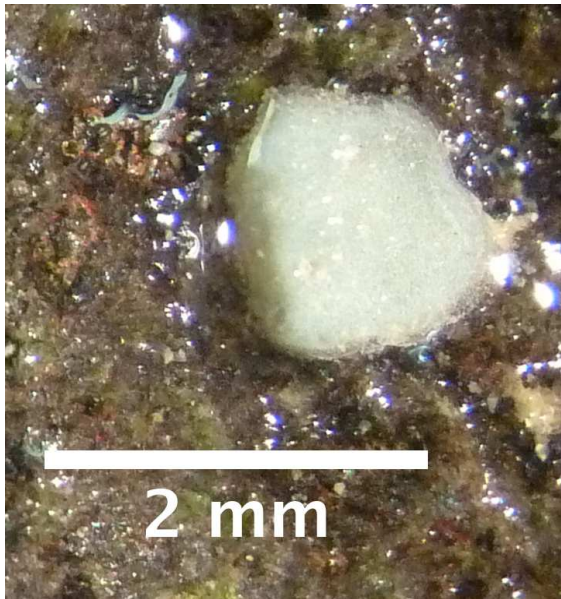Adult, *Neritina turrita*, collected on October 18th 2013.

FIG. 1. *Nerita argus:* single egg capsule

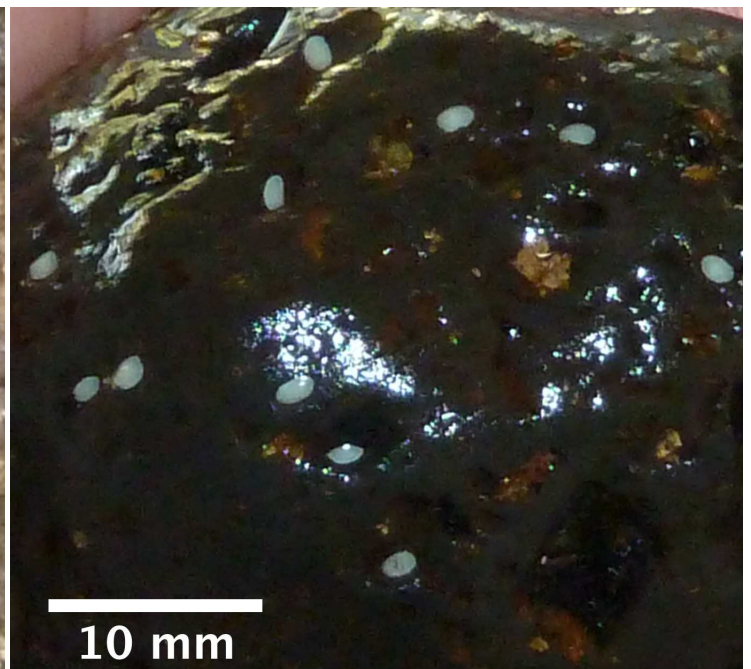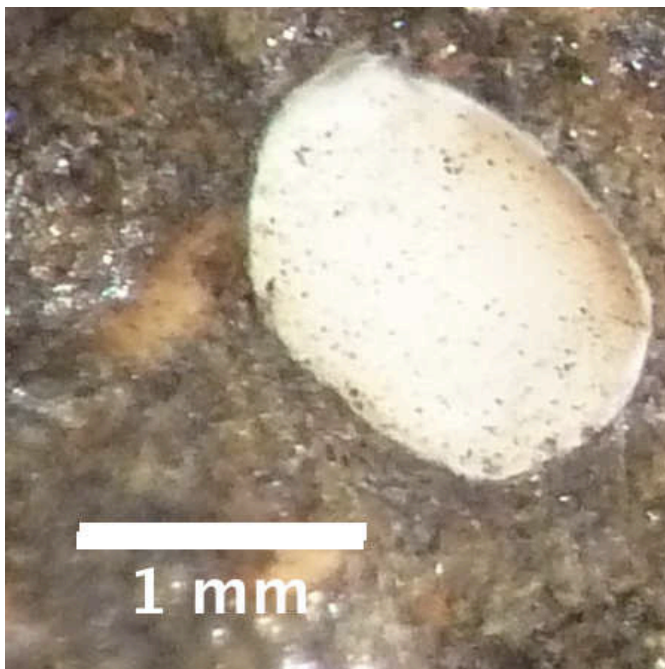FIG. 2. *Neritina canalis:* batch of egg capsules

FIG. 3. *Neritina turrita*: egg capsule (left) and batch of capsules (right)

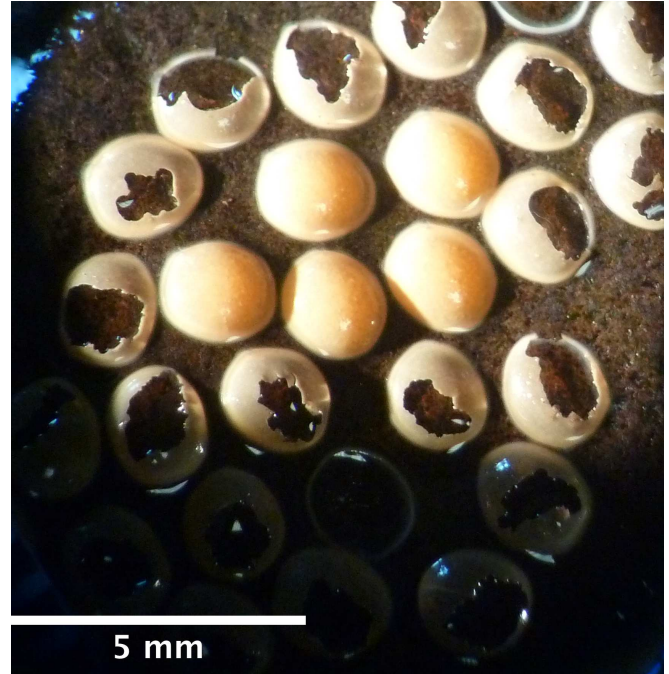FIG 4 unknown ID (perhaps very old *S. porcellana* capsules*)*

FIG 5 *Septaria porcellana*: egg capsules, both hatched and unhatched.

FIG 6 unknown ID

APPENDIX C

FIG. 1. *N. argus*: freshly laid egg capsule on rock

FIG. 2. *N. argus*: underside of egg capsule that was removed from rock, embryos less than one day old

FIG. 3. *N. argus:* Egg capsule that has been removed for over 10 days and has embryos that have formed eyespots
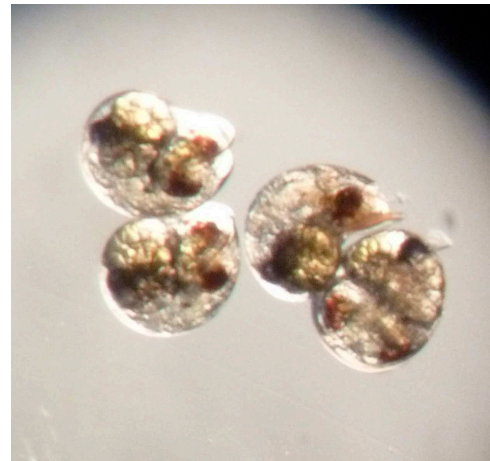

FIG. 4. *Nerita argus*: Fully developed veliger larvae

APPENDIX D

TABLE 3. Fecundity in captivity of neritid snails and the sites where they were collected. Eggs were laid between October 25th and November 18th. n=number of individuals collected from that site.

	Nerita argus	N. turrita		N. canalis	
site	Opunohu	Afareaitu	Opunohu	Afareaitu	Opunohu
n	35	6	8	45	1
per capita fecundity	**2.83**	**0**	**14.75**	**0**	**0**
total fecundity	**99**	**0**	**118**	**0**	**0**

	Septaria porcellana		Spinosa	
site	Afareaitu	Opunohu	Afareaitu	Opunohu
n	3	10	0	6
per capita fecundity	**0**	**0**		**0**
total fecundity	**0**	**0**		**0**

TABLE 4. Fecundity at Opunohu and Afareaitu during surveys where eggs were scraped clear of rocks and then reexamined 10 days later.

Canalis		turrita		S. porcellana	
Afareaitu	Opunohu	Afareaitu	Opunohu	Afareaitu	Opunohu
0	0	0	153	0	0