Possible anti-predation properties of the egg masses of the marine gastropods *Dialula sandiegensis, Doris montereyensis* and *Haminoea virescens* (Mollusca, Gastropoda)

E. Sally Chang[1,2]

Friday Harbor Laboratories
Marine Invertebrate Zoology
Summer Term 2014

[1] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250
[2] University of Kansas, Department of Ecology and Evolutionary Biology, Lawrence, KS 66044

Contact information:
E. Sally Chang
Dept. of Ecology and Evolutionary Biology
University of Kansas
1200 Sunnyside Avenue
Lawrence, KS 66044
Eschang1@gmail.com

Keywords: gastropods, nudibranchs, Cephalaspidea, predation, toxins, feedimg, crustaceans

Abstract

Many marine mollucs deposit their eggs on the substrate encapsulated in distinctive masses, thereby leaving the egg case and embryos vulnerable to possible predators and pathogens. Although it is apparent that many marine gastropods possess chemical anti-predation mechanisms as an adult, it is not known from many species whether or not these compounds are widespread in the egg masses. This study aims to expand our knowledge of egg mass predation examining the feeding behavior of three species of crab when offered egg mass material from three gastropods local to the San Juan Islands. The study includes the dorid nudibranchs *Diaulula sandiegensis* and *Doris montereyensis* and the cephalospidean *Haminoea virescens.* The results illustrate a clear rejection of the egg masses by all three of the crab species tested, suggesting anti-predation mechanisms in the egg masses for all three species of gastropod.

Introduction

Eggs that are laid and then left by the parents are vulnerable to a variety of environmental stressors, both biotic and abiotic. A common, possibly protective strategy among marine invertebrates is to lay encapsulated aggregations of embryos in jelly masses (Pechenik 1978), where embryos live for all or part of their development. It has been suggested that the function of the egg masses is one of protection against the hazards of being free-floating while in early stages of development (Pechenik 1979).

For the gastropods, cost of producing the egg cases appears to be substantial and requires specializations in the adult gastropod, so there must be some protective or other benefit (Pechenik 1979). For example, it has been suggested that these egg cases provide protection from ultraviolet light, but this may have more to do with placement with respect to the environment of the egg masses by the adult gastropods than the structure of their egg cases (Biermann et al. 1992). One major suggestion about the benefit of egg encapsulation is that it provides protection from predators that would otherwise eat non-motile masses of embryos (Pechenik 1979). Egg cases do seem to be subject to predation by many groups of benthic animals, including other molluscs and crabs (Roche et al. 2011), as well as fish and polychaetes (Pechenik 1979, citing D'Asaro 1970).

The heterobranch gastropods provide a fascinating look into the development of anti-predation defenses. Members of the Nudibranchia are known to be toxic, and development of these toxins is thought to correlate with the reduction or loss of the shell in nudibranchs and other opisthobranchs (Faulkner and Ghiselin 1983). For example, nudibranchs of the Family Doridacaea in general are studied for both toxic and non-toxic compounds of interestthe biomedical industry.and many of the secondary metabolites that can be traced back to the prey sponge (Walker and Faulkner 1981). Nudibranchs can both synthesize defensive chemicals de novo or acquire them from their sponge diet, depending on species, and may depend on the local food available to the nudibranch or how specialized the diet of the nudibranch is (Faulkner et al. 1990).

Some muricid gastropod egg masses have been shown to have anti-fouling properties, due to chemical defenses (Ramasamy et al. 2007), and many molluscan egg masses have been shown to have antibacterial properties (Benkendorff et al. 2001). Some recent evidence indicates that protective compounds can be passed on to the egg cases. In a study of the Spanish dancer nudibranch (*Hexabranchus sanguinous*) some of the secondary metabolites found in the adult nudibranch are also found in extracts from the egg ribbons (Pawlik et al. 1988).This study was undertaken in order to expand our knowledge of the palatability of heterobranch gastropod egg cases by conducting feeding experiments using local crustaceans and the egg masses of local gastropods. Ideally, these experiments will shed light on one potential facet of the adaptive significance of these structures – that is, their ability to deter predation — and improve our understanding of how widespread these defenses are among the gastropods.

Study system

In this study, I tested the palatability of the egg cases of two species of nudibranchs and one species of cephalaspidean:

Doris montereyensis J.G. Cooper 1863 (Mollusca:Gastropoda); known as the false sea lemon or the Monterey dorid. *Do. montereyensis* (Fig. 1) is an adorable, primarily yellow dorid nudibranch with black spots on the dorsum that are present on the dorsal tubercles. *D. montereyensis* primarily eats the encrusting yellow sponge *Halichondria panicea* (Bloom 1981). This species is found from Alaska to San Diego, CA (Behrens and Hermosillo, 2005), and is easily accessible from collecting sites on the San Juan Islands. *D. montereyensis* lays a yellowish, rose-shaped collection of fluttering egg ribbons (Behrens and Hermosillo, 2005). Extracts made from adult *Do. montereyensis* have been demonstrated to be toxic to crabs and mice (Fuhrman et al. 1979).

Diaulula sandiegensis J.G. Cooper 1863 (Mollusca:Gastropoda). A whitish yellow to pale brown dorid nudibranch with a cream to light brown dorsum, blotches or dougnut-shaped rings on the dorsum (Fig. 1). This species can be found from the Aleutian islands, to Baja California, as well as on the coast of Japan. It is a relatively generalized sponge feeder (Behrens and Hermosillo 2005). *Di. sandiegensis* deposits relatively widely spaced rings of white fluttering egg ribbons. The flesh of *Di. sandiegensis* has been shown to have acetylenes possibly derived from the sponges they consume (Walker and Faulkner 1981), although it appears there have not been any studies on whether or not those compounds are also present in the egg cases.

Haminoea virescens Sowerby II 1833 (Mollusca:Gastropoda). A bubble shell cephalaspidean found between Alaska and Panama. *H. virescens* has green skin with yellow spotting and a small, exposed shell that can be opaque to pale green (Behrens and Hermosillo 2005) (Fig. 1). Several other members of the genus *Haminoea* appear to possess secondary metabolites used as alarm pheremones located in the cephalic shield, parapodial lobes and the posterior pallial lobes (Marin et al. 1999).

Four crabs of three different types were used as the feeding subjects for this experiment: two individuals of the Tanner crab, *Chionocetes bairdi* Rathbun 1924 (Arthropoda:Crustacea), one individual of the graceful decorator crab *Oregonia gracilis* Dana 1851 (Arthropoda:Crustacea), and one individual of the sharpnose crab *Scyra acutifrons* Dana 1852 (Arthropoda:Crusacea) (Fig. 2). All of these crabs have been described as generalist predators or scavengers (Jewett and Feder 1983, Hines 1982) and have so far appeared to eat anything given to them in our sea tables, making them suitable candidates to test whether or not something is palatable to potential consumers.

Materials and Methods:

This experiment was designed to see whether or not the egg cases of *Di. sandigiensis*, *Do. montereyensis* or *H. virescens* would be eaten by the various species of crab as compared with a control food. Overall, crabs were subjected to a total of four feeding treatments: one for the egg case of each species, and one control. The control food was made of two packets of Knox Gelatine mixed to double strength with 2 grams of tetramin food powder mixed in, and allowed to cool into a thin sheet. Samples and control for the experiment were designed to have roughly the same dimensions in order to eliminate possible size-related effects on the crab's feeding choice behavior. The ribbons of the gastropod egg cases were cut up to roughly standardized pieces, about 2.5 by 1.25 cm, and the control sheets were cut up into 'cookies' of roughly the same dimensions. These cookies were readily eaten by a variety of crustaceans in a general laboratory sea table.

Commented [DKP1]: need to spell out the *D.*'s -they mean different things here!

Due to the unwillingness of the crabs to eat while kept in isolated tanks, the experimental feeding had to take place in the main sea table, without isolating the animals from other potential predators or enforcing starvation prior to the feeding trials. Animals had not been deliberately fed prior to the experiment, but it was not possible to completely eliminate edible material from the environment. Each piece of control or egg material was presented to a particular test crab with long forceps and dropped in such a way that they made contact with the crab's body.

Four rounds of the experiment were performed with each crab. Each round consisted of offering the crabs the samples one by one, with a twenty minute break after each round. To minimize the effects of the order in which the crabs were offered the different treatments, the treatments were given to the crabs in a different order each round. One control feeding was done per round.

Each trial within an experimental round was scored as simply "sample eaten" or "sample not eaten." If the animal had not eaten the food within fifteen minutes, this counted as not eaten. Likewise, tasting and manipulation with an eventual rejection counted as not eaten. If the crab held on to the sample and ate the sample for over ten seconds without any rejection behavior, this was scored as eating. Crabs were observed for as much of the experiment as possible to assess behavior responses.

Because the results for each experimental round for each crab were identical, these results were collapsed into whether or not a particular crab ate a particular treatment. A G-test was used in order to determine whether there was an association between being either a control sample or egg sample and whether or not the sample was eaten.

Results

None of the egg mass samples, from any of the gastropods, were consumed by any of the crabs during the experiment. This was in direct contrast with the control, which the crabs ate every single time it was placed in front of them. There was no difference between species of crab or species of gastropod which produced the egg mass being tested. A G-test with 3 degrees of freedom was highly significant with a test statistic of 17.99 and a p-value of less than .001 (see Table 1 below and Figure 3 for details).

Table 1. Summary of results of the feeding trial, broken down by sample type. This data was the raw input for the G-test.

Sample Type	# of Crabs that ate sample	# of Crabs that did not eat
Tetramin “cookies”	4	0
Di. sandiegensis egg case	0	4
Do. montereyensis egg case	0	4
H. virescens egg case	0	4

Several distinct behavioral responses were noted repeatedly throughout the trial. When one of the crab’s appendages came in contact with the egg material, it would be brought to the mouth. Shortly thereafter, the crab removed the egg mass from the mouth with one of the chelipeds, and pushed it away from their body. In other cases, the crab would touch the tips of their legs to the piece of egg case, but not ever bring the piece towards their mouth.

Discussion

The results of this experiment clearly display the inability or unwillingness of the subject crabs to consume fragments of egg masses from the marine gastropods *Di. sandiegensis, Do. montereyensis* and *H. virescens* As mentioned above, there was no difference in the results between the different species of crabs that were fed. These results provide some support for the hypothesis that the egg masses of these gastropod species possess some sort of anti-predation properties.

This work does not touch on the mechanisms or causes behind the apparent aversion of the crabs toward the gastropod egg cases. One possibility is that the egg cases possess some form of anti-predation compounds. For both *Di. sandiegensis* and *Do. montereyensis,* studies have shown that the adults have potentially toxic secondary metabolites possibly derived from the sponges that they eat (Walker and Faulkner 1981). *Di. sandiegensis* has also been shown to produce some of these compounds de novo (Kubanek and Anderson 1999). Extract from the body of adult *Do. montereyensis* has been demonstrated as lethal when injected into mice and shore crabs (Fuhrman et al. 1979). In a study of the Spanish dancer nudibranch (*Hexabranchus sanguinous*) some of the secondary metabolites found on the adult nudibranch are also found in extracts from the egg ribbons (Pawlik et al. 1988), indicating that these compounds may be passed to the egg masses by adult gastropods. However, it is also possible that the thickness and material properties of the egg case, rather than toxic compounds, prevented the crabs from feeding on the material. One studyhasreported on the tensile strength and tenacity of gastropod egg masses attached to their substrate (Castro and Podolsky 2012), but not much is known about their mechanical resistance to predation.

The distinct rejection behavior by the crabs in most of the trials may indicate that it was not a simple inability to cut open the egg masses that prevented the crabs from eating the samples. A previous study of crustacean feeding behavior noted stereotypical behavior similar to what was observed in this experiment as crabs chose whether or not to consume a particular piece of food (Lee and Meyers 1996). According to Lee and Meyers (1996), crustaceans exhibit four major categories of behavior prior to feeding: antennule flicking, probing movements made by the pereopods towards the food, locomotion by the crustacean, indicating true attraction or repulsion; and movements by the mouthparts that indicate generalized feeding stimulation. The crabs in this experiment clearly demonstrated some initial attraction and locomotion towards the egg mass samples, but this was usually followed by quick movement of the piece away from their body, or vice versa, which seems to indicate a deliberate and rapid choice of the crabs to not consume the egg masses.

This study provides an initial look at the possible anti-predation defenses present in several local species of marine gastropods (*D. sandiegensis, D. montereyensis* and *H. virescens)*, and provided evidence of the unpalatability of these egg cases, at least to decapod crustaceans. It will be important to test the egg cases of these gastropods against other predators, especially since it appears other marine gastropods prey on these cases (Pechenik 1979, Roche et al. 2011). The discovery of anti-predation chemicals should help facilitate the search for similar adaptations in other marine gastropods, so that the overall distribution and frequency of these defenses can be studied.

More experiments about the exact origin and nature of the defense will also prove to be useful. For example, it is unknown whether the putative toxins are secreted only by the adult onto the mass, or are produced throughout development by the embryos themselves. Explorations of the toxicity or palatability of the mass through developmental time and with the embryos removed may prove useful for understanding the adaptive role of these defenses. In general, this paper provides convincing evidence that there is some predation defense in the egg masses of local gastropods, and should prove the starting point for future research in this area.

Acknowledgements

The author would like to thank everyone who helped her complete this work. Drs. Dianna Padilla and Michael LaBarbera provided generous assistance, both in terms of laboratory assistance and intellectual assistance. Special thanks to Dr. Dianna Padilla for help with overall experimental design and set up. The course TA Kevin Turner also provided valuable insight to the particulars of the animals in this study and statistical advice. Thanks also to the emotional and intellectual support from my fellow Invert. Zoo. classmates.

I would also like to thank my sources of funding that made my attendance at Friday Harbor Laboratories possible. I received an Ida H. Hyde Scholarship through the University of Kansas Department of Biology. From FHL, I was supported by the Chris Reed Fellowship, as well as an Adapt-a-Student fellowship. I would like to give a big thank you to my sponsors Veronique Robigou and Bruce Nelson.

Finally, I would like to acknowledge the nudibranchs collected for this study for being cooperative participants.

Literature Cited make all titles in the same style

Anson, H. Hines. (1982). Coexistence in a Kelp Forest: Size, Population Dynamics, and Resource Partitioning in a Guild of Spider Crabs (Brachyura, Majidae). Ecological Monographs, 52(2), 179-198. doi: 10.2307/1942610

Benkendorff, K., Davis, A. R., & Bremner, J. (2001). Chemical defense in the egg masses of benthic invertebrates: An assessment of antibacterial activity in 39 mollusks and 4 polychaetes. Journal of Invertebrate Pathology, 78(2), 109-118. doi: 10.1006/jipa.2001.5047

Bloom, S. A. (1981). Specialization and noncompetitive resource partitioning among sponge-eating Dorid nudibranchs. Oecologia, 49(3), 305-315. doi: 10.1007/bf00347590

Rudman, W.B., 2006 (February 14) Eastern Pacific Nudibranchs. A guide to the Opisthobranchs from Alaska to Central America . David W. Behrens & Alicia Hermosillo. *[In] Sea Slug Forum.* Australian Museum, Sydney. Available from http://www.seaslugforum.net/factsheet/brbehrherm

Castro, D. A., & Podolsky, R. D. (2012). Holding On to a Shifting Substrate: Plasticity of Egg Mass Tethers and Tethering Forces in Soft Sediment for an Intertidal Gastropod. Biological Bulletin, 223(3), 300-311.

Faulkner, D. J., & Ghiselin, M. T. (1983). Chemical defense and evolutionary ecology of Dorid nudibranchs and some other opisthobranch gastropods.Ecology Progress Series, 13(2-3), 295-301. doi: 10.3354/meps013295

Fuhrman, F. A., Fuhrman, G. J., & Deriemer, K. (1979). Toxicity and pharmacology of extracts from Dorid nudibranchs.. Biological Bulletin, 156(3), 289-299. doi: 10.2307/1540918

Jewett, Stephen C., & Feder, Howard M. (1983). Food of the Tanner Crab *Chionoecetes bairdi* near Kodiak Island, Alaska. Journal of Crustacean Biology, 3(2), 196-207. doi: 10.2307/1548256

Kubanek, J., & Andersen, R. J. (1999). Evidence for de novo biosynthesis of the polyketide fragment of diaulusterol a by the northeastern Pacific Dorid nudibranch *Diaulula sandiegensis*. Journal of Natural Products, 62(5), 777-779. doi: 10.1021/np9804839

Lee, P. G., & Meyers, S. P. (1996). Chemoattraction and feeding stimulation in crustaceans. Aquaculture Nutrition, 2(3), 157-164. doi: 10.1111/j.1365-2095.1996.tb00055.x

Marin, A., Alvarez, L. A., Cimino, G., & Spinella, A. (1999). Chemical defence in cephalaspidean gastropods: Origin, anatomical location and ecological roles. Journal of Molluscan Studies, 65, 121-131. doi: 10.1093/mollus/65.1.121

Pawlik, J. R., Kernan, M. R., Molinski, T. F., Harper, M. K., & Faulkner, D. J. (1988). Defensive chemicals of the Spanish dancer nudibranch *Hexabranchus sanguineus* and its egg ribbons – macrolides derived from a sponge diet.Journal of Experimental Marine Biology and Ecology, 119(2), 99-109. doi: 10.1016/0022-0981(88)90225-0

Pechenik, J. A. (1979). Role of encapsulation in invertebrate life histories.. American Naturalist, 114(6), 859-870. doi: 10.1086/283533

Ramasamy, M. S., & Murugan, A. (2007). Fouling deterrent chemical defence in three muricid gastropod egg masses from the Southeast coast of India. Biofouling, 23(4), 259-265. doi: 10.1080/08927010701278857

Roche, A., Maggioni, M., & Narvarte, M. (2011). Predation on egg capsules of *Zidona dufresnei* (Volutidae): ecological implications. Marine Biology, 158(12), 2787-2793. doi: 10.1007/s00227-011-1777-5

Walker, Roger P., & Faulkner, D. John. (1981). Chlorinated acetylenes from the nudibranch *Diaulula sandiegensis*. The Journal of Organic Chemistry, 46(7), 1475-1478. doi: 10.1021/jo00320a046

Figure 1. The gastropods used in this study and their egg cases. From top, *D. sandiegensis, D. monteryensis and H. virescens*. Photos of *D. sandiegensis* and *D. montereyensis* eggs as well as adult *H. virescens* were taken from http://www.seaslugforum.net. All other photos by author.

Figure 2. Crustacean predators used in experiment. From top *S. acutifrons, C. bairdi* and *O. gracilis.* All photographs by author.

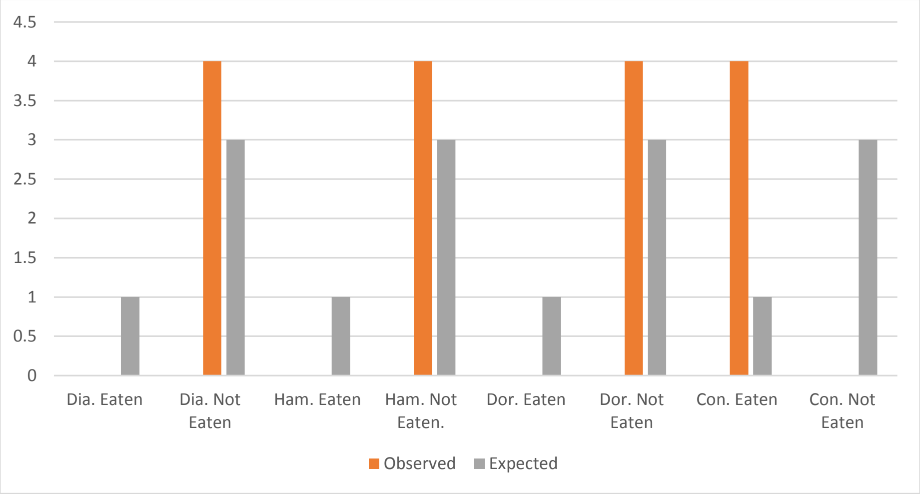

Figure 3. Comparison between expected and observed values for eat vs. did not eat categories for each sample type, as calculated as part of the G-statistic. Dia = *D. sandiegensis,* Ham=*H. virescens* and Dor=*Doris montereyensis.*