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# Protection from the Effects of Ultraviolet Radiation by the Encapsulating Structures of Embryos of Physa, a Freshwater Pulmonate Snail

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## Recommended Citation

# Protection from the Effects of Ultraviolet Radiation by the Encapsulating Structures of Embryos of *Physa* sp., a Freshwater Pulmonate Snail

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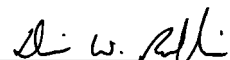**Illinois Wesleyan University at Bloomington, Illinois**

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**Senior Honors Committee Members**

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**Abstract**

The genus *Physa* includes freshwater pulmonate snails that inhabit shallow environments well within depths penetrated by ultraviolet (UV) radiation. The distribution patterns of egg masses of *Physa* sp. indicate that the snails lay egg masses in sunlit areas and the masses are attached to rocks and debris such as leaves and twigs. Each mass consists of a viscous jelly covering that encases a variable number of embryos, and each of the embryos is individually surrounded by an egg capsule. Field-collected masses were found to have debris and epifaunal organisms attached to the jelly. Preliminary studies of the effect of UV radiation on the developing embryos of these snails indicated that embryos surrounded by the capsule and jelly coat experienced no detriment from UV exposure. However, removal of the jelly coat from encapsulated embryos and subsequent exposure to UV radiation resulted in a >90% mortality. The UV absorption of the jelly coat was measured in both laboratory-raised and field-collected egg masses using a UV-Vis spectrophotometer. Absorption of energy by the jelly covering was between the wavelengths of 275-300 nm. This is consistent with absorption of UV-B radiation. The fact that no difference was detected in absorbance between field and laboratory egg masses suggests that the jelly is providing protection, rather than any organism or material adhered to the egg jelly.

**Introduction**

The effects of ultraviolet (UV) radiation (UV-B 280-320nm, UV-A 320-400nm) on development and the mechanisms by which the embryos repair the damage have been well documented in a number of organisms (Hoval and Morgan 1999, Nagl and Hofer 1997, Rawlings 1996, Carefoot *et al.* 1998). Often repair mechanisms are not established until mid-blastula stage (see Epel *et al.* 1999), but even then, they are not always one hundred percent effective in reversing the damage. In early embryos with totipotent cells, the damage of one cell is almost certainly life threatening so prevention by minimizing exposure to UV radiation is preferred. Prevention can take several forms

such as the timing of spawning, the place of spawning, the buoyancy of the gametes, and/or the presence of protecting materials (Epel *et al.* 1999, Epel 2003).

While many studies have examined the preventative measures an organism uses against UV radiation, such as the incorporation of mycosporine-like amino acids and antioxidants within the egg (Carefoot *et al.* 1998, Sutherland *et al.* 2003), little has been done on the role of extra-embryonic structures as a protective covering for embryos. A study by Rawling (1996) reported that *Nucella emarginata*, a marine benthic gastropod, uses encapsulation of the developing embryo to limit exposure to UV radiation. The encapsulating jelly of the tadpole larvae of five amphibian species has also been shown to absorb UV light (Smith *et al.* 2002). In addition, a species' photolyase activity (a mechanism to repair UV damage) inversely correlates with the jelly absorbance of UV at 320nm. Therefore, if the embryo cannot repair the damage, its jelly has the ability to absorb more of the harmful radiation and therefore prevent damage from occurring (Smith *et al.* 2002).

The genus *Physa* includes freshwater pulmonate snails that inhabit shallow environments well within depths affected by UV radiation. The snails lay egg masses that are attached to the substratum. Each mass consists of a viscous jelly covering that encases a variable number of embryos each of which is individually surrounded by a capsule. As in *Lymnaea stagnalis* (Wijsman *et al.* 1987), the gelatinous covering of *Physa* sp. egg masses has two distinct regions: a tough outer membrane (tunica capsularis) and an inner, more fluid jelly (tunica interna) (Figures 1,2). The embryos undergo larval-like development within the capsules and pass through stages resembling the trochophore and veliger larval stages typical of other molluscs (Dewitt 1954).

This study reports the distribution pattern of egg masses of *Physa* sp. *in situ* to determine whether the adults oviposited in sunlit areas and therefore exposed the developing embryos to UV radiation. Absorption spectra of jelly masses were examined to determine the UV radiation absorption capacity of the jelly. Additionally, field-collected and laboratory raised masses were compared in their ability to absorb UV radiation to determine if epifaunal organisms, which naturally grow on the surface of the jelly, provide additional protection Finally, a protocol for determining intra-mass hatching variability was refined for use in future experiments.

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## Materials and Methods

*Position of egg masses in the field*

  Egg mass placement was measured in a shallow portion of the Mackinaw River (40°40'N, 39°W) , Kappa, Illinois in September, 2003. The area observed measured 136.7 m$^2$ and followed the shoreline of an island where the current was slow relative to the water current of the main river flow. The water depth ranged from two to seven centimeters and had a bottom composition of sand and mud. All rocks and debris (leaves and wood) were picked up and examined for the presence and position of egg masses (top, side or bottom) and then removed from the transect area. Approximately 30 adult snails and 20 egg masses that were attached to rocks and debris were placed in plastic containers and transported to Illinois Wesleyan University.

*Snail upkeep*

  Adult snails used for obtaining laboratory-laid egg masses were placed into glass dishes in groups of 4-6 individuals with filtered (0.8μm pore size) freshwater. The containers were covered with cling wrap and placed in a Percival incubator (Model #I-30BL) at 25°C with a 12/12 photoperiod. The snails were fed Noyes Precision Food pellets every other day. Old food and feces were removed every other day to reduce bacterial growth, while a complete water change was provided every four days. Egg masses were removed from the walls of the dishes with a razorblade and collectively placed in a glass dish with filtered (0.2μm pore size) freshwater (from here on after to be referred to as filtered freshwater).

*Capsule removal*

  Laboratory-laid and field-collected egg masses were individually placed into a small glass dish with a minimal amount of filtered freshwater. A 22-gauge needle was attached to plastic tubing which was in turn connected to a vacuum. A clamp was placed on the plastic tubing to fine-tune the strength of the suction. Viewed through a Meiji EMZ dissecting microscope, an egg capsule was manipulated with micro-forceps to the

edge of the mass, and the needle with gentle suction was used to tease it from the jelly. Even with the clamp, the vacuum was too high and sucked up too much jelly so a new method was developed. The suction was eliminated, but the dissecting mircoscope, needle and micro-forceps were still used. The micro-forceps were placed behind an egg capsule and the needle pulled the capsule up the shaft of the forceps until it broke free from the mass. Jelly loss still occurred, however, the amount lost decreased with the new method.

*Spectrophotometry*

In a preliminary study, combined jelly and capsules of *Physa* sp. were tested in their ability to absorb UV radiation. Four jellies containing capsules were placed into a 2mL quartz cuvette and ran against a blank of filtered freshwater in a Cary 1E UV-Vis spectrophotometer. Several runs were performed with the same cuvette to determine whether sinking of the jellies with capsules affected the absorbance curve.

Egg masses were grouped into four categories: laboratory-raised whole egg mass (jelly, capsule, and embryo), laboratory-raised jelly only, field-collected whole egg mass, and field-collected jelly only. For each group, 2-3 masses were combined into a 200µL quartz cuvette (light path length 12mm, width 2mm) and evaluated in their ability to absorb UV light using a Shimadzu UV-Vis spectrophotometer (Model UV-1601) against a blank of filtered freshwater. A minimal amount of filtered freshwater was added to keep the masses/jelly suspended. Each grouping of entire mass or jelly was analyzed for absorbance three times; the cuvette was shaken after each analysis. For the laboratory-raised jelly, two groups of 2-3 masses was run three times each, so the total number of spectra created was six. Only one group of field-collected jelly was evaluated and run three times; the total number of spectra created was three. UV probe software by Shimadzu was used to create the spectra, and then that data was transferred to Microsoft Excel.

*Determining a protocol for the estimation of intra-mass hatching variability*

A Logitech web camera was connected to a Toshiba laptop computer that ran ImageStudio 7.0.0. Dishes containing the egg masses were placed on plastic platform

that was placed on a light table. The web camera was situated above the light table with the use of a ring stand. A pair of polarized filters was used to illuminate the shells and therefore easily track hatching. One filter was placed between the light table and the dish containing the egg masses, and the other filter was attached either to the web camera or directly on top of the container. The light table continually illuminated the egg masses, and time-lapse photography was used (1 frame/5 minutes).

Egg masses were placed in different types of containers to determine which set-up facilitated the estimation of hatching times. The containers included small-diameter deep wells and shallow plastic Petri dishes. The Petri dishes were used when egg masses were laid on Cling Wrap and could be easily handled. The egg masses were placed in the Petri dish with the Cling Wrap towards the bottom and filled with 0.8um filtered freshwater. The small wells were ideal when a mass was free-floating (*i.e.* it was removed from the walls of the adult dishes).

The time-lapse videos were checked at various points throughout the day. To determine hatching times, the frame in which the first snail was found to have hatched was labeled zero. Every frame after that was then counted and each time a new hatching occurred, the frame number was multiplied by five minutes (the time between frames) to determine how much time passed between subsequent hatchings. This system provided precise time of hatching, allowing for a comparison of hatching time within and among egg masses.

**Statistical Analyses**

Using standard procedures of Chi-square analysis found in Zar (1999), the distribution of egg masses on leaves, rocks, and wood was analyzed. Table 1 illustrates the number of masses found in each position (top, side, bottom) and the substrata on which they were found (leaves, rocks, wood). Position of masses on the substrata was divided into two groups: masses that received light, and masses that did not receive light. Masses found on the sides of rocks and wood were divided equally into the two groups, since they are only exposed to direct sunlight a portion of the day. The three categories

(top, side, and bottom) were then compared against each other to determine if a more specific relationship existed between ovipositing and position on a substratum

## Results

*Egg mass placement*

Both a laying preference in substrata and position on the substrata was detected ($p < 0.05$). The number of egg masses found in sunlit areas was significantly greater than the number of egg masses found in areas that did not receive sunlight. The number of egg masses found on the bottoms of the substrata was significantly less than the numbers found on the sides and tops. The number of masses on the tops and sides, however, did not significantly differ. This supports the previous analysis that adult snails show a preference for ovipositing in areas that received sunlight.

When the numbers of masses found on each type of substratum (rock, leaf, and wood) were compared to each other, it was found that there were significantly less egg masses on leaves then on wood and rocks. Detecting no significant difference between the numbers found on wood and rocks suggests that the adult snails are laying on more stable substrata.

*UV absorbance of field-collected vs. laboratory-raised masses*

The six laboratory jelly spectra and three field jelly spectra were each averaged. The average values were then subtracted by their starting value at 400nm (the lower cutoff of visible light) to normalize the data so both spectra start with zero absorbance at 400nm. With decreasing wavelengths, an increase in absorption is seen with a single maximum at ~280 nm, which is in the range of UV-B radiation and where proteins containing aromatic amino acids absorb. To determine whether the differences in graphs was merely quantitative (the concentration of the substances were different), the difference of normalized averages of field and laboratory jellies was taken at one data point (249nm – one nm below the cutoff point of our spectrum). The value that came from the difference was then subtracted from the remaining values (250nm-330nm) of the field jelly spectrum (Figure 3). The resultant spectrum was similar to the laboratory jelly

spectra, meaning no new peaks appeared. The differences between lab and field jelly were merely quantitative, and the epifaunal organisms that inhabited the field-collected jelly provided no detectable protection from UV radiation.

The field and laboratory jelly spectra was then compared to the preliminary spectrum of the whole-mass jelly and capsules. The preliminary spectrum shows the same pattern of absorption, with decreasing wavelength, an increase of absorbance is seen with a single broad maximum at ~280 nm (Figure 4). The absorbance of this spectrum, however, is much lower than the field and laboratory jelly-only spectra. This is likely due to the different sized cuvettes used. With the 2mL cuvette, the concentration of the material was much less than the total volume of the cuvette, compared to the 200μL cuvette, creating smaller net absorbance readings.

*Determining a protocol for the estimation of Intra-mass hatching variability*

Determining intra-mass hatching variability was easiest when the egg mass was attached to cling wrap. If the mass is left to float freely, even in a small space, the mass moves too much, making it difficult to determine hatching times. Smaller spaces also make it difficult to determine hatching times with larger masses because of the crowding exhibited by hatchlings. The newly hatched snails have limited space and therefore stay near the egg mass, making it difficult to observe when a hatching occurs.

The cling wrap/Petri dish combination was not successful because the light table caused rapid evaporation of water overnight that led to desiccation of the egg masses. The refractive properties of the plastic sometimes made it difficult to observe the masses.

Placing the cling wrap with the attached egg masses in a large amount of water works best. If the cling wrap is placed into a larger glass dish, the egg mass remains in one position, and the hatchlings have more room to move away from the mass, making determination of hatching times more accurate. Depending on the memory capabilities of the computer, and the degree of accuracy wanted, the time-lapse can be lengthened or shortened, or a continuous-stream video can be run.

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## Discussion

The preference of *Physa* sp. to oviposit in areas receiving sunlight suggests that either preventative or repair mechanisms have been evolved to protect their embryos from the harmful effects of UV radiation. Other studies show species of aquatic invertebrates laying on the undersides of substrata (Reich *et al.* 2003) and, therefore, unexposed to UV radiation. While laying on the undersides of substrata may protect from UV radiation and desiccation, light may be necessary for successful development for some aquatic species for several reasons. Light, through the production of heat, is known to increase metabolism and therefore the rate of development (Belehradek 1935 *in* Hoegh-Guldberg and Pearse, 1995). This potentially decreases the time developmental stages spend immotile. By decreasing the time spent in developmental stages, an organism can reach reproductive maturity more quickly (see Thomas 1990). Decreasing the time spent in an immobile state may also decrease predation, however, for *Physa* sp. and other species of molluscs, the encapsulating structures actually decrease predation (Pechenik 1986).

As an additional preventative measure, members of *Physa* sp. may lay at night. For *Physa fontinalis*, it was found that laboratory-reared adults oviposit between midnight and 8am, thus protecting early cell divisions from UV radiation (Duncan 1959). Another possibility is that the water contains high concentration of dissolved organic carbon (DOC) that attenuates UV radiation, especially UV-B, before it reaches the embryos (Nagl 1997). The depth at which egg masses of *Physa* sp. were found (2-7cm), it is unlikely that DOC plays a significant role as a protection for the snails. The jelly mass, which has long been thought of as a structure to prevent desiccation and possibly predation, (Epel *et al.*, 1999, Pechenik 1986) is now being looked at as a structure capable of absorbing UV light.

Preliminary studies have shown that the encapsulating structures of *Physa* sp. (the jelly and capsules) can absorb UVB light (Nielsen *et al.* unpub. data). These studies, however, failed to discriminate which structure provided the primary protection. By measuring the absorbance of the jelly of laboratory-collected egg masses and then

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comparing it to the spectrum of the jelly and capsule absorbance, it was found that the capsules provide no apparent additional protection. This study is further supported by the findings of Brennan *et al.* (unpub. data). Encapsulated embryos were irradiated for 20 minutes with 30 Watt UVB bulb (280-320nm) with and without the jelly and the percent mortalities were compared. Embryos irradiated with all extra-embryonic structures intact showed no significant difference in the number of mortalities than with the unirradiated control. Removal of the jelly coat with subsequent irradiation however resulted in a >95% mortality.

The comparison of absorbance of field-collected and laboratory-raised jelly suggests that epifaunal organisms that are naturally found attached to the surface of the jelly, do not provide significant additional protection against UV radiation. Future studies need to determine the amount of UV radiation egg masses receive *in situ* to assess the effects UV light has on the development of encapsulated embyos of *Physa* sp., with and without the jelly mass. Furthermore, the composition of the jelly needs to be determined to understand how the jelly provides protection.

## Acknowledgements

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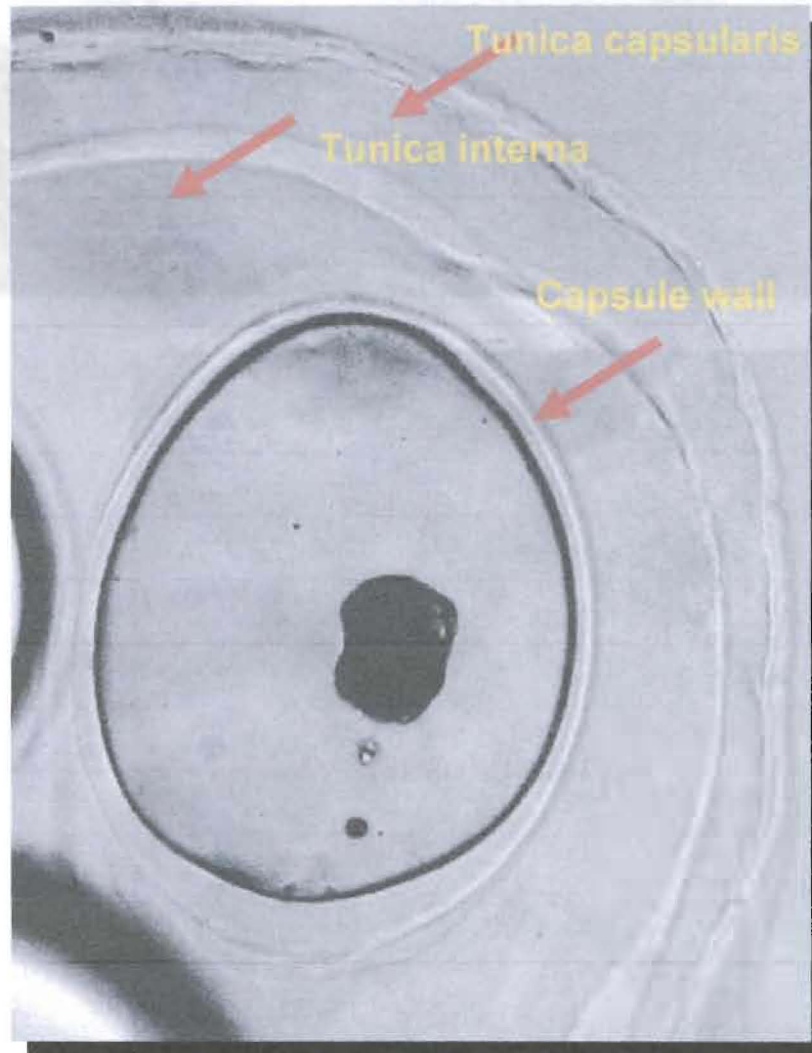
Figure 1: Light Microscopy Photograph of a two-day old embryo illustrating the
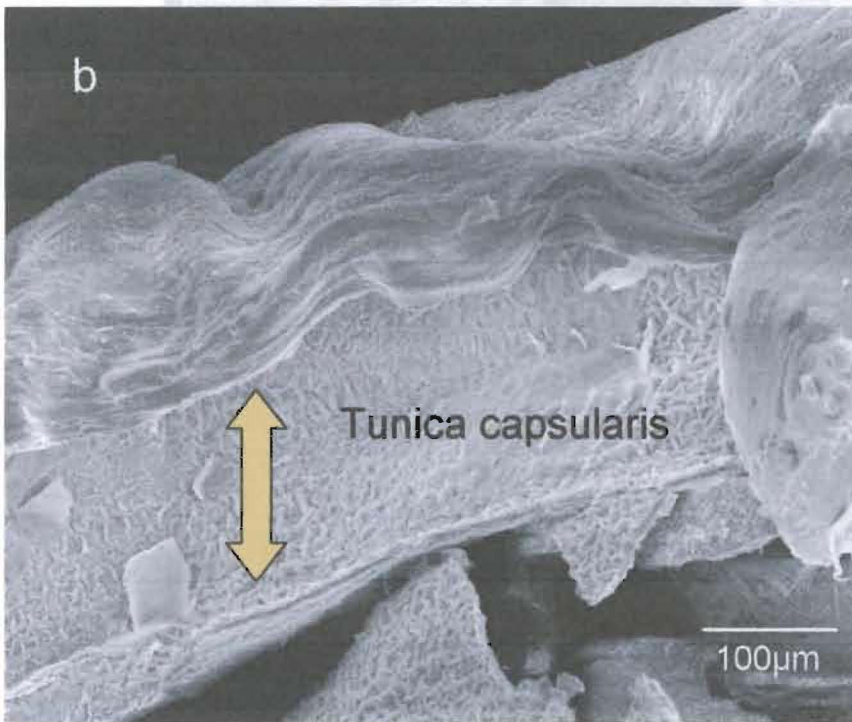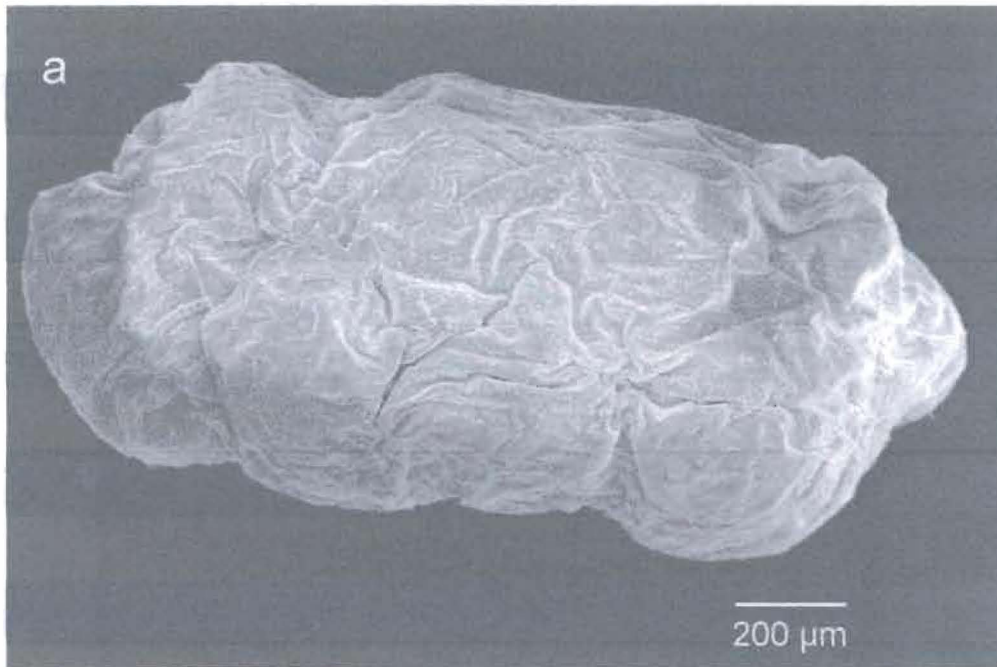components of the extraembryonic structures.

Figure 2:  Scanning electron micrographs of egg masses of *Physa* sp. using standard preparation protocol (Balser and Jaeckle unpub. data). (a) Entire egg mass (b) Outer layer of jelly coat with the inner layer broken up below.

|            | Egg Mass Position |      |        |
| ---------- | ----------------- | ---- | ------ |
| Substrate  | Top               | Side | Bottom |
| Wood       | 5                 | 24   | 13     |
| Leaf       | 17                | 0    | 1      |
| Rock       | 23                | 9    | 2      |

Table 1: The data table created from the transect showing the number of egg masses on each type of substratum and their position on the substrata.
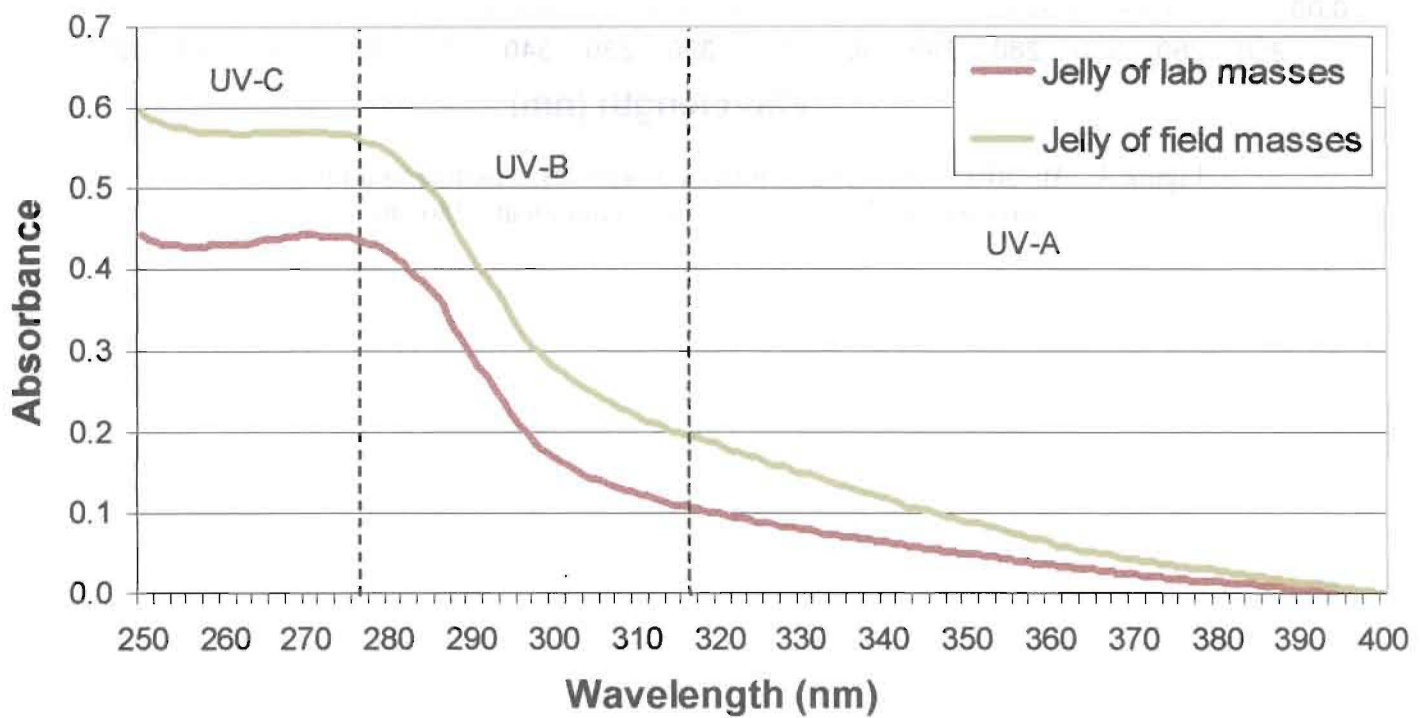


Figure 3: Absorbance of both lab jelly and field jelly is plotted as a function of wavelength. Notice the single maximum of both spectra at ~280 nm.
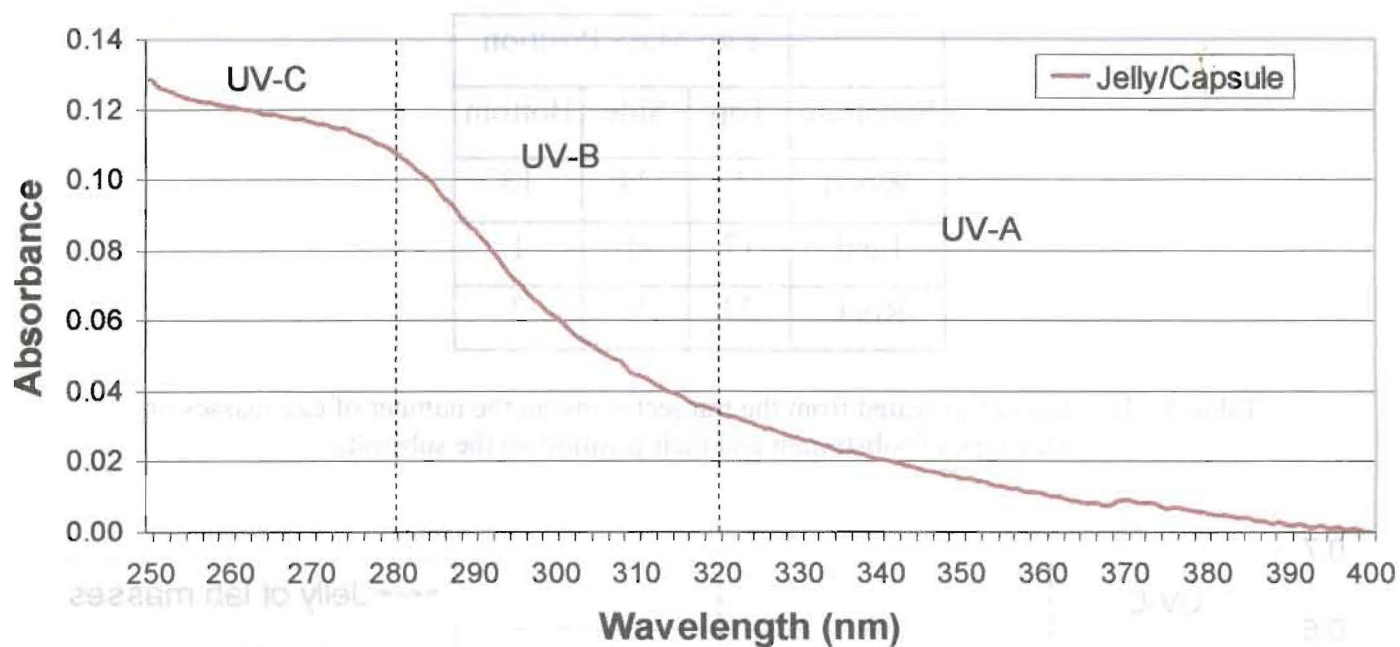
Figure 4: Absorbance of both the jelly and capsules is plotted as a function of wavelength. Notice the single maximum at ~280 nm.