Desiccation Rates and Thermal Tolerance in *Collisella digitalis* and *Tectura persona*

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Adaptations of Marine Animals

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Introduction

In the rocky intertidal off of the Oregon coast there are marked vertical zonation patterns. This phenomenon exists in this area due to the intertidal fluctuations that occur every day, extreme tidal amplitudes that create very different conditions in the different vertical zones. There are three distinct zones intertidal: the supra-littoral zone, the mid-littoral zone and the intra-littoral zone. The organisms living in the supralittoral zone are submerged for all but the lowest of the low tides, and are adapted to a life almost exclusively in the water. Conversely, the animals living in the intra-littoral zone are exposed for all but the highest of high tides, and are adapted to a life built around living in the air. The upper limit for many organisms is set by their ability to cope with physiological or biochemical factors such as desiccation, temperature fluctuations and the exposure to direct sunlight. Organisms who are unable to deal with these factors are excluded from life in the upper intertidal, and are found lower where the physical pressures are not as great.

There are two species of limpets that can be observed living on the dry rocks of the upper intertidal: *Collisella digitalis,* which is commonly known as the finger limpet, and *Tectura persona,* known as the masked limpet. Desiccation is a major facto in the upper intertidal, and the two limpet species share an important common factor that helps them avoid the intense desiccation risk that faces them in the dry intertidal. "A factor of...significance in determining desiccation rates is the ability of both *C. digitalis* and *T. persona*, to form a sheet of mucus between the margin of the shell and the substrate (Wolcott 1973). This layer of mucus secreted between the shell and the substrate was stated by Wolcott to be the most important adaptation in terms of limiting desiccation, indicating that the two limpet species lose water at similar rates, allowing them to survive long periods without water.

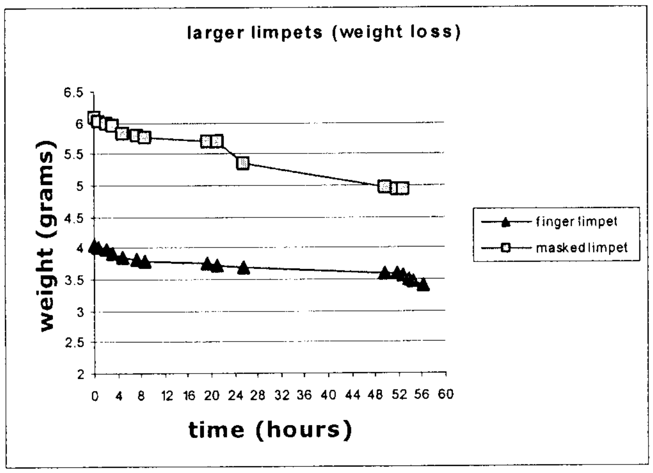
Yet *C. digitalis* and *T. persona* are found in very different microhabitats in the upper intertidal, indicating that adaptive differences between the two species must exist. *C. digitalis* is most often found on vertical rocks, located in areas receiving little or no protection from direct solar radiation and yet even on the hottest days, this species does not retreat from thermal pressures associated with such direct exposure. *T. persona*, on the other hand, copes with the intense sun exposure in the upper intertidal by avoiding it. "*T. persona* is a large, smooth olive shelled limpet, found in dark crannies and under boulders, sheltered from the sun. It is negatively phototaxic, moving and feeding only at night (Wolcott, 1973)." And indeed, on warm sunny days, if one ventures out into the intertidal, many masked limpets can be seen clustered in dark crevices escaping the heat. It is thus hypothesized that *T. persona* is less able to cope with the thermal pressures associated with the upper intertidal than is *C. digitalis,* and that it is this vulnerability rather than a greater sensitively to desiccation that causes it, but not the finger limpet, to retreat from the sun.

Materials and Methods

Three specimens of both *C. digitalis* and *T. persona* were collected off of the rocks in the high intertidal from South Cove, Cape Arago. The animals were removed using a metal knife, separating the limpets cleanly from their substrates and inflicted no damage to their outer shells. Limpets that did not easily come away from the rock were not collected as the limpets were usually injured upon subsequent attempts at removal (Wolcott, 1973). The limpets collected fell into one of three categories: Large, medium and small. The size classes were defined in relation to each other, i.e. the larger limpets were larger than the small and medium limpet classes. The collected population included two large limpets, two medium limpets and two small limpets, with each size class containing a *C. digitalis* and a *T. persona* specimen. The limpets were kept in a aerated saltwater table in a lab setting for one day after they were collected from the field, ensuring that none of the limpets were experiencing any desiccation effects prior to the experiment and as an attempt to reduce stress due to their prior removal from their natural setting. The limpets were kept in a plastic container with a mesh top. After this initial acclimation period, the limpets were removed and weighed individually using a digital scale. Any excess water on the shell or body of the limpets was removed with a paper towel. The shell was thoroughly dried, and the foot was lightly blotted. Each limpet was then placed onto a pre-weighed lid of a plastic petri dish where it then established suction. These were then placed in the direct sunlight during the day for three consecutive days. They were moved inside during the nighttime and early morning hours to avoid accumulation of rain or dew condensation that would affect the rate of desiccation. During this time period the masses of the limpets were recorded 16 different times (the intervals between these measurements were rather sporadic due to a lack of staff at the time of the experiment). Any mass lost during the experiment was assumed to be primarily water mass in the form of water-based bodily fluids. The behaviors of the limpets were also monitored and recorded through out the experiment. For each limpet, measurements were taken until they were no longer attached strongly to the petri dish surface. It was assumed that the animals were ecologically (if not also physically) dead at this point as in the wild such a limpet would be unable to resist either the pull of the tide or predation pressures (Wolcott 1973). The percent of water lost from the initial bodily amount was calculated using the following equation: (1 - (final weight)/(initial weight)) * 100%, and the total amount of mass lost was calculated by subtracting the final weight from the initial weight.

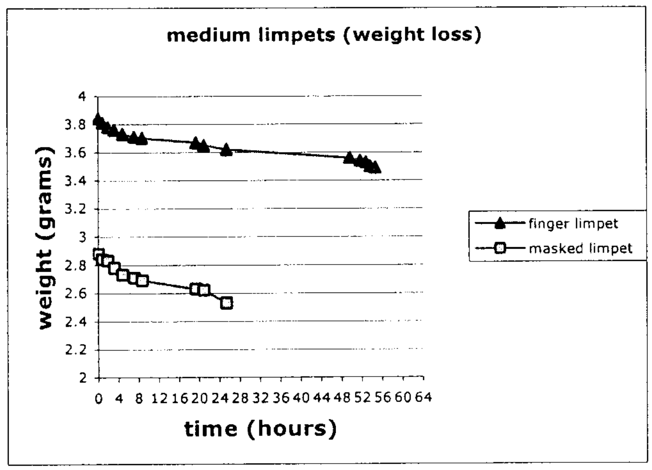
<u>Results</u>

Figure 1:



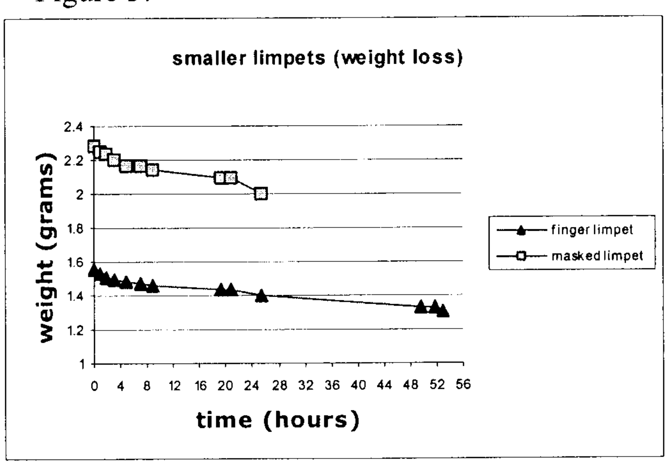
% Body weight lost at time of death:
Large *C. digitalis* : 16 %
Large *T. persona:* 18 %
Total amount of mass lost at time death:
Large *C. digitalis*: .64 g
Large *T. persona* : 1.1 g

Figure 2:



% Body weight lost at time of death:
Medium *C. digitalis* : 10 %
Medium *T. persona*: 12 %
Total amount of mass lost at time death:
Medium *C. Digitalis*: .35 g
Medium *T. persona*: .35 g

Figure 3:



% Body weight lost at time of death:
Small *C. digitalis* : 10 %
Small *T. persona:* 11 %
Total amount of mass lost at time of death:
Small *C. digitalis*: .16 g
Small *T. persona* : .25 g

Discussion

As hypothesized, the two limpet species desiccated at very similar rates during the course of the experiment, a similarity attributed to the common formation of a mucus lining between the shell and the substrate, which was observed for both limpets species. As can be seen in figures 1, 2 and 3, the limpets in each size class lost water mass at very similar rates. Unexpectedly, the larger limpets of both species initially desiccated at a slightly faster rate than did the smaller limpets. This difference can perhaps be attributed to a greater amount of free water trapped under the shells of the larger limpets upon their initial weighting and suction to the petri dishes. This water would evaporate faster than would the water housed in the tissue of the extra-visceral mass, and would cause to larger limpets to lose water weight more quickly in the beginning when they were exposed to air. However, The larger limpets of both *C. digitalis* and a *T. persona* were able to sustain more water loss overall before they died than the two smaller classes of both species. This indicates that a larger size is a beneficial attribute when water loss is occurring; larger animals can lose a greater amount of water before the loss becomes fatal, allowing them to survive desiccating conditions for a longer amount to time as opposed to smaller limpets which experience fatal consequences associated with proportionally less water loss.

However, at one point in the experiment, the desiccation rates began to differ between *T. persona* and *C. digitalis.* Due to their very a different reactions to the temperature conditions under which they were maintained. It is this behavioral difference between the two limpets species which supports the hypothesis that *T. persona* is less able to cope with thermal stress than *C. digitalis.* After 20 hours and 54 minutes had elapsed (a time period that included 1:45 on a sunny afternoon), all three of the masked limpets were observed to have lifted their shells up off of the plastic substrate to which they were attached. Suction continued to be maintained, but at a much weaker strength than had been observed only 4.5 hours prior. This change in position was not observed in any of the finger limpets, the shells of all three *C. digitalis* limpets remained in contact with the substrate to which they were suctioned. At this time, their level of suction remained strong and their attachment to the plastic firm. During the time that the masked limpets separated their shells from the plastic substrate the rate of desiccation observed increased dramatically, ultimately causing the masked limpets to reach the lethal level of desiccation much sooner than the finger limpets in the corresponding size categories. Between 20.9 hours and 25.4 hours into the experiment, (during which the masked limpets had separated their shells from the plastic) all of the *T. persona* specimens lost significantly more water mass than had been observed previously. The large *T. Persona* lost 5.1 times more water mass per hour during this time period, and the medium and small *T. persona* respectively lost 1.6 and 1.8 times more per hour. Further more, only the larger masked limpet survived this initial heat increase, lowering its shell back flush with the substrate over night when conditions cooled. The two smaller classes of *T. persona* presumably died due to heat exposure, which also initiated a behavioral reaction that accelerated the process of evaporation and thus desiccation. Even the large *T. persona* was ultimately unable to completely recover from the consequences of this intense and rapid water loss, or from the destruction of the protective mucus lining. The large *T. persona* died 5.3 hours before the correspondingly large *C. digitalis*. The Finger limpets never reached their temperature limit in the daytime sun and thus were not observed lifting their shells up to cool off. They instead died primarily due to factors associated solely with desiccation (excessive loss of internal water).

Previous research has indicated that when limpets are sequestered from water and exposed to factors that promote desiccation they will eventually exhibit this behavior and "raise their shells off of the substrate. When the animal raises its shell, the extra-visceral mass is presented to the air. Evaporation, and, therefore, cooling, may now take place (Segal and Dehnel, 1962). Later, Wolcott elaborated observation stating "Under desiccating conditions the limpets tend to conserve water rather than using it to regulate body temperature; the shell-lifting response is probably evidence of impending heat coma (1973). It can thus be concluded that *T. persona* more than *C. digitalis,* when forced to stay in contact with direct sunlight, is in a greater danger of overheating to the point of death. It then follows that *T. persona's* survival in the upper intertidal is primarily limited by thermal stress, a factor to which *C. digitalis* has been shown to be more suited.

The conclusions drawn from this experiment can be strengthened by observing the limpets in the upper rocky intertidal. *C. digitalis* can be found on vertical rock surfaces, completely unprotected from the sun and its influences. Perhaps this ability to tolerate high thermal condition can be related to the morphology of the shell of *C. digitalis,* which is covered in ribbing which extends down from the apex. In other organisms, such as the California mussel, extensive ribbing on the shell is thought to reflect sunlight, a protective measure against a potential increase in temperature. The ribbing may play the same role in *C. digitalis,* permitting its exposure to the direct sunlight, explaining why the smooth shelled *T. persona* is excluded. The conclusions of this experiment are also supported by the locomotory behavior displayed by the masked limpet in it natural environment. As mentioned in the introduction, *T. persona* is negatively phototaxic, sensing and actively moving away from light from the sun and into crevices that protect it from direct solar radiation. This behavior can be further explained by current research which indicates that there is a significant temperature gradient between crevices and exposed rock surfaces in the rocky intertidal: "Rock temperatures and limpet body temperatures were cooler by up to 12° C in crevice habitats compared with exposed habitats (Gray and Hodgson, 2004)." *T. persona*'s preference for such crevices most likely indicates a preference for the lower temperatures found there, supporting the hypothesis that the masked limpet is less able to cope with thermal stress than is the finger limpet, a species often found exposed to the sun and thermal extremes.

There are several directions that this research could be taken to further understand the physiological and biochemical factors that determine which environmental pressures *T. persona* and *C. digitalis* can tolerate. For example, it would be interesting to see if the thermal tolerance of *C. digitalis* would change if the ribbing on the shell was completely removed. If *C. digitalis* showed a lower tolerance to thermal extremes, the ribbing on the shell could be assumed to be the quality that currently allows it to stay in the sun while the smooth *T. persona* must retreat to cooler crevices. Another interesting question is whether the two limpets express differing levels of heat shock proteins, which are chaperone proteins that prevent other proteins from denaturing due to a temperature increase. It is possible that *C. digitalis* simply expresses more of these proteins than *T. persona,* which is why it can tolerate exposure to higher temperatures. Either of these two avenues of research would greatly expand what is currently known about the different reactions of the masked limpet and the finger limpet to the thermal stresses that exist in the upper rocky intertidal.

Works Cited:

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