Peter Tierney
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# The effects of thermal stress on *Nucella lamellosa* and *Lottia pelta* radula

ABSTRACT:

Radula formation in gastropods has been shown to be sensitive to the environmental conditions, including substrate type and low temperatures. However, little work is available on the effect of high temperature stresses on rate of radula development. This study is a first-order attempt in examining the relationship of radula production and temperature between two gastropod species with very different uses for their radula—a predator, *Nucella lamellosa,* and a grazer, *Lottia pelta*—under elevated temperature conditions. Row density of new radula growth, and relative tooth wear along the anterior end of the radula, were compared between species and across 3 temperature settings. A negative correlation between water temperature and row density was observed for both study species. Furthermore, elevated temperature was negatively correlated with both feeding rates and tooth wear in *N. lamellosa*. This study suggests that stress from elevated temperatures is detrimental to radula development between species, and that temperature stress leads to a reduction in feeding, and thus, reduced usage of the radula.

## INTRODUCTION:

Throughout their lives gastropods in the intertidal are subjected to huge temperature fluctuations. Although they are adapted to wide variation in temperature, there are upper and lower temperature limits that these organisms can tolerate, and local adaptation selects for those points of highest stress. One key to studying adaptation in local populations is to understand the effect of these stresses and to seek effects that leave a record. Hard parts such as shells fit well into this paradigm, as they are continuously accreted, and in many organisms they may grow in different manners based on

environmental conditions, including temperature (Kennish & Olsson, 1975). However, shells accrete very slowly, and may not present the temporal resolution necessary to record events that last only a day or two. In order to identify such periods, a finer resolution record would be very useful.

One particular avenue for finding this resolution would be gastropod radula. They are produced throughout the lives of gastropods at a rather rapid pace, many growing at a rate of 2-3 rows per day (Padilla et al., 1996). As rows of teeth at the anterior end of the radula are worn down through feeding, they are shed, only to be replaced by new rows formed at the posterior end. Rows may be added to the radula at a rate of several rows per day, the exact rate varying between species, and perhaps conditional on ontogeny (Fujioka, 1985). Some workers suggest that the rate of radula replacement decreases with increasing age or shell size, especially in gastropods with richoglossan radula, such as most neogastopods (Meirelles & Matthews-cascon, 2003).

The production of radula in many gastropods has been shown to be detrimentally affected by low temperatures. For example, Fujioki (1985) found that in Japanese Nucellids, *Thais bronni* and *Thais clavigerus*, radula is rarely produced below 10°C, and when it is, it is strikingly thin and malformed. This is not a phenomenon limited to those particular species, but appears to be common among many gastropod species at different temperatures (Isarankura & Runham, 1968). Fujioki (1985) also noted alteration of radula production on the other end of the temperature scale; in these Nucellid species, radula production and turnover increased with higher temperatures. Alternatively, species of marine pulmonate gastropods have shown a decrease in radula production when exposed to high temperature stresses (Smith & Russell-Hunter, 1990).

This study attempts to examine the effect of elevated temperatures on radula growth on two species found around San Juan Island: the predatory snail, *Nucella lamellosa* and the grazing limpet, *Lottia pelta*. The aim of the study is twofold: 1.) to examine the nature of gastropod radula growth in

response to temperature, including the rate of production, row density, and eventual use, and 2.) to examine whether or not there is any difference in response to elevated temperature between species that live in the same part of the intertidal at the same location, but with different feeding habits.

METHODS:

49 whelks (*Nucella lamellosa*) and 66 limpets, presumed to be monospecific (*Lottia pelta*), were collected from Eagle Cove, on the southern shore of San Juan Island. Each individual was acclimated to the holding tanks at Friday Harbor Labs for two days. The organisms were then placed in a refrigerator and kept at 4°C for roughly 17 hours in order to induce cold-shock in the radula. Afterwards, the organisms were returned to ambient seawater conditions for duration of a day to recover from cold-shock.

Three temperature regimes were sustained in 5-gallon plastic tanks. One tank remained at the ambient temperature of the seawater flowing into the labs (11-12°C). The second tank was maintained at lab air-temperature (17-18°C), and the third was kept at 22-23°C with the aid of handheld, immersable aquarium heaters. In each tank was placed a supply of food for the study organisms, including at least 260 small barnacles (primarily *Balanus glandula*) for *N. lamellosa* and 25 g of brown algae (*Laminaria* sp.) for *L. pelta*. Numbers of living barnacles and weight of algae will be recorded prior to placement in the tanks. The two populations will be divided equally between the three tanks and acclimated to the desired temperature over the course of two days.

After spending five days in the tanks, post-acclimatization, the gastropods in the control tank were prepared for dissection in 7% dilute Magnesium Chloride solution. In order to stagger dissections while limiting further radula growth, all specimens were prepared at once, with specimens preserved for later dissection in refrigerated containers. On the $6^{th}$ day in the tanks, the experimental groups were removed from their tanks and prepared for dissection similarly.

The dissection involved the removal of the radula from the odontophoral cartilage. The radula will be dried in 50% dilute ethanol, then shifted to a 95% concentration. Samples were mounted and sputter coated with gold palladium for imagine with a scanning electron microscope. For each radula, total length (in rows) and rows added since cold shock, if applicable, were documented before preparation for SEM analysis. In addition, measured length of the radula and length of the newest 20 rows were also recorded. Length of the radula was not taken if the radula was split during dissection, however, row counts were still taken if all pieces of the radula were retained. Notes on population changes (relatively increased feeding, or mortality) were taken in conjunction and used in analysis.

In order to characterize wear on radula, a simple method was devised. The radula of *N. lamellosa* contains multiple teeth, among which are three primary teeth, and adjacent inner secondary teeth (Fig. 1B). Rows of radula from the anterior end were counted until a row with remaining secondary teeth could be distinguished. For *L. pelta*, a similar method was used, but rather the key feature to identify was the sharp points on the ends of their radular teeth (Fig 1A). At least three samples were observed within the SEM for each group of organisms in each tank. For all six groupings, the length of worn radula was characterized, but not critically assessed considering the potential loss of fragile anterior rows during dissection.

RESULTS:

Upon completion of the trial runs, expired individuals from each tank were removed. The control group had 5 *L. pelta* expire, the tank at 17°C lost only 2 *L. pelta* individuals, and the 22°C tank lost 6 *N. lamellosa* and 7 of the collected *L. pelta*. The trials were interrupted by a 6-hour power outage, which left the tanks without the aquarium pumps and heaters, so temperature was not consistent over the entirety of the experiment as well. Furthermore, in the interest of time, dissections were limited to 17 *L. pelta* and 9 *N lamellosa* from the control group, and only 5 samples from each of the remaining

four groups. This count does not include specimens removed from analysis due to cases of mistaken species identification. At least two specimens were later identified as *Tectura scutum*, and replaced in the study.

The dissected radula failed to consistently record a cold shock, perhaps due to a temperature far higher than the 1-2C used in other studies (Fujioka, 1985; Isarankura and Runham, 1968) . No interruption in row development could be identified in the majority of specimens. No attempt at analysis of rates could be made. A summary of radula lengths, and the comparison of density of new rows to density of older rows can be found in Tables 1 and 2.

The groups did not consistently eat during the experiment. The brown algae in the 17°C tank was pitted and torn by *L. pelta*, suggesting feeding, and the total weight of the remainder was 4.5 g less than what was initially placed in the tank. No difference in weight or appearance could be found in brown algae placed in the control tank, and the algae placed in the 22°C tank decayed and became gelatinous. There was no sign of feeding on the algae before removal. In contrast, *N. lamellosa* showed feeding in all tanks through their activity as well as in the count of barnacles post-experiment. After 5 days, 164 fewer living barnacles could be found in the control tank. After 6 days, 110 and 131 fewer barnacles could be found in the 17 and 22°C tanks, respectively.

In the analysis of wear, *N. lamellosa* consistently showed significant wear up until the $14^{th}$ row from the anterior end in the 3 specimens observed. Among radula prepared for SEM from the 17°C tank individuals, wear was observed to rows 11-13, and in the 22°C tank, rows 11-14. Among *L. pelta*, radula wear was observed to rows 4-5 in both the control group and in the 17°C tank. In the 22°C tank, wear was observed until rows 5-6.

DISCUSSION:

The organisms in the tanks showed inconsistent activity that could suggest high levels of stress, including reduced feeding among *N. lamellosa*. *L. pelta* feeding rates could not be fully determined as individuals in the control and 22°C tank may have eaten algal growth on the aquarium walls instead of the food provided. Moreover, the mortality rates in the highest temperature tank can be considered a result of high thermal stress, at least in *N. lamellosa*. The mortality rate in *L. pelta* was only marginally larger than in the control group, so it may not be reasonable to suggest death was likely a heat-stress response. Causes of stress in the tanks could include not only temperature, but other events during the experiment. For example a blackout cut oxygenation in the experimental tanks for at least 6 hours. In addition, the constant prodding and removal of dead organisms may have brought on further stress among the others in the tank.

Measurements did not present significant differences in average shell length, or radula length between the experimental and control groups. There was an apparent increase in radula length in *N. lamellosa* between the control and experimental groups, but the average radula length of the experimental groups still fall within expected standard deviation of the control. A reduction in feeding is expected to lengthen the radula because the rows at the anterior end will not be shed in use, but the slight increase in radula length contradicts the slight decrease in the number of rows observed among the groups kept at elevated temperatures.

Row density at the posterior end of the radula visibly increased between the control groups and the experimental groups. In comparison to normal row density of the remainder of the radula, the rows created during the experiment were far more compact. However, the calculated shift in row density between the older part of the radula and its most recent 20 rows did not fall outside the expected standard deviations of the control group. A larger sample size would be needed to determine whether the alteration of row density could be significant. Notably, very little change in row density is apparent

between the two experimental groups. Although it is apparent, through increased mortality, that the organisms in the 22°C tank were highly stressed, there does not seem to be a large increase in row density. In comparison between organisms at each temperature setting, it appears *L. pelta* row density remains more consistent in production between ambient and elevated temperatures than *N. lamellosa*. Radula rows produced by *N. lamellosa* are visibly compressed in all groups until the part of the radula in which full mineralization takes place. Thus, the inconsistency in *N. lamellosa* row density between the control and experimental groups could either be a factor of the small sample size or may reflect a stress response in early radula production. Further analysis may also look into the structural integrity of the rows produced under elevated temperatures, post mineralization, as this study was not long enough to produce fully mineralized rows in most specimens.

Much like the lack of clear trends between temperature and the row density of new radula growth, no consistent trend can be found between radula wear in either group. Only in the experimental groups did radula of *N. lamellosa* show radula wear to fewer than 14 rows from the anterior end. Although this characterization is from a small sample size, the difference could reflect the reduced feeding rates observed in the experimental groups of *N. lamellosa*. New rows will move up with shedding on the anterior end, but the unworn rows would not be used as often, thus retaining the secondary teeth addressed with the methodology of this study. Under the same rationale, though, the increase in wear among *L. pelta* radula at the highest temperature settings would also reflect an increase in feeding, but this could not be corroborated with the data, as there was no visible marking on the algae placed in that particular tank. If eating was not consistent, it is likely that rows of radula were not removed consistently, and thus new, unworn rows would fail to move up in position and impact the characterizations created in this study.

In summary, there is not a significant difference observable in row density, radula length or wear between the organisms in each temperature setting. There may be some differences in radula production between the two species, though, with *N. lamellosa* showing a stronger increase row density under high temperatures than *L. pelta*. The reasoning for this is still unclear. This study did not observe a definite response to temperature in the growth record of radula, but more work is certainly needed to check for different types of responses, such as alteration of growth rates or a weakening of the tooth structure under stress.

Future work may include a second approach to this experimental setup in order to readdress the initial question of radula growth rate. In addition, further studies may address geographic variation in growth responses to elevated temperature. It is known among other species of *Nucella* that temperature tolerance can vary significantly throughout a species' geographic range (Kuo & Sanford, 2009). With its benthic juveniles, flow between populations of *N. lamellosa* is limited and thus, location-specific adaptation is a possibility. In comparison, *L. pelta* disperses more freely as a swimming veliger larvae, and thus might show a greater uniformity in tolerance and response between locations due to increased population connectivity.

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## TABLES AND FIGURES:

Table 1: Summary statistics for *Lottia pelta* radula. Note the decrease in radula density ratio between the control group and those in elevated temperatures.

| Temperature Setting | No. of Rows | Radula Length (mm) | Length of Final 20 Rows (mm) | Ratio of Row Densities--Last 20 Rows / All Else |
|---|---|---|---|---|
| 12°C | 61 ± 3 | 19.0 ± 3.0 | 6.2 ± 0.9 | 0.98 ± 0.06 |
| 17°C | 62 ± 4 | 18.9 ± 3.1 | 5.6 ± 0.7 | 0.9 ± 0.03 |
| 22°C | 60 ± 3 | 17.8 ± 3.7 | 5.6 ± 1.0 | 0.93 ± 0.04 |

Table 2: Summary statistics for *Nucella lamellosa* radula. Note decrease in radula density ratio between the control and the experimental groups

| Temperature Setting | No. of Rows | Radula Length (mm) | Length of Final 20 Rows (mm) | Ratio of Row Densities--Last 20 Rows / All Else |
|---|---|---|---|---|
| 12°C | 273 ± 47 | 14.7 ±1.7 | 0.925 ± 0.125 | 0.85 ± 0.22 |
| 17°C | 274 ± 17 | 15.1 ± 1.1 | 0.72 ± 0.04 | 0.64 ± 0.02 |
| 22°C | 270 ± 28.7 | 15.7 ± 0.6 | 0.76 ± 0.13 | 0.64 ± 0.08 |

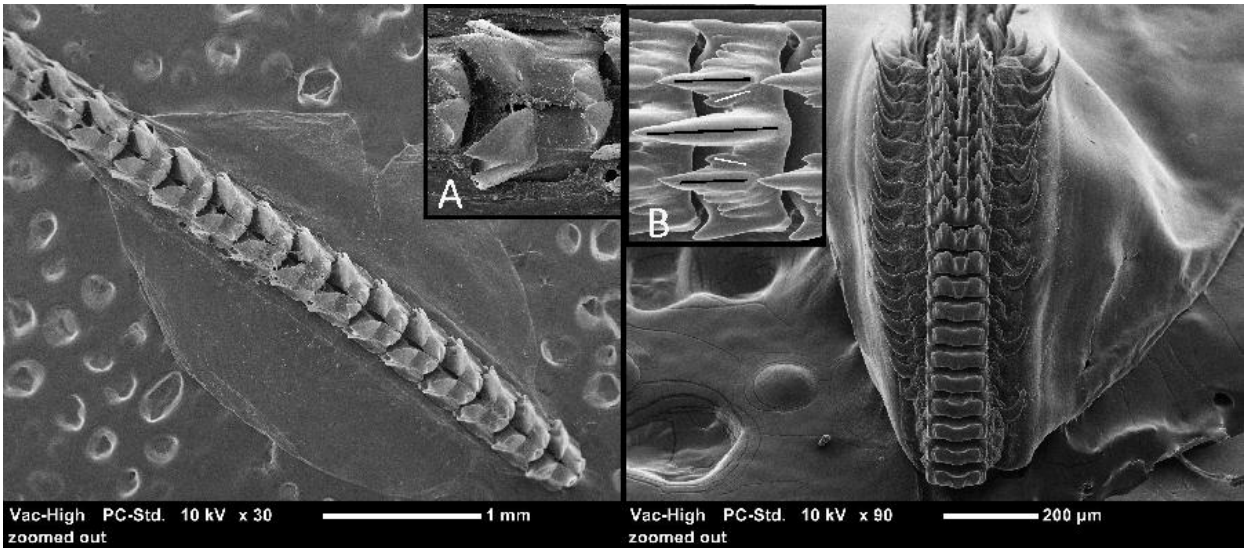


Figure 1: Radula of *Lottia pelta* (A) and *Nucella lamellosa* (B). Note the organization of the teeth, including the sharp edges in *L. pelta* teeth and the primary (black line) and secondary (white line) teeth in *N. lamellosa*. Rows were counted from the anterior end until the features were visible.