J Moll. Stud. (1996), 62, 275–280

RADULAR PRODUCTION RATES IN TWO SPECIES OF *LACUNA* TURTON (GASTROPODA: LITTORINIDAE)

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(Received 1 September, 1995, accepted 20 November 1995)

ABSTRACT

The molluscan radula is a dynamic organ, both in terms of its use and production. New rows of teeth are constantly produced at the posterior end of the radula, while older, worn teeth are shed anteriorly, producing a dynamic equilibrium. We used a cold-shock to mark the radular ribbon and measure tooth row production rates in two gastropod species, *Lacuna vincta* (Montagu) and *L. variegata* Carpenter. We found that the average tooth row production rate at 10–11°C did not differ between these two species, and was 2.94 (SE = 0.002) rows per day for *Lacuna vincta* and 2.97 (SE = 0.002) for *L. variegata* Inter-individual variability in production rate was very low, and was correlated with shell length, smaller individuals had slightly higher production rates. The total length of the radular ribbon varied greatly among individuals, ranging from 47 to 94 (2.57 to 5.68 mm) rows in *L. vincta* and 53 to 99 rows (2.80 to 7.14 mm) in *L. variegata*, and was only somewhat correlated with the length of the shell. This great variability will result in large differences among individuals in the time it takes to replace the radula totally, from 14.96 to 35.44 days in *L. vincta* and from 17.43 to 39.69 days in *L. variegata*.

INTRODUCTION

The radula is a dynamic structure, both in terms of its use and its production. The radula consists of a long ribbon of tissue with repeated rows of teeth along its length. Teeth can be chitinous, proteinaceous, or may be mineralized with, for example, iron or silica (Lowenstam, 1962; Runham & Thornton, 1967). The shapes of radular teeth are important for determining the effectiveness of the radula as a tool for feeding (Padilla, 1985, 1989). Because teeth become worn as they are used, which can affect their shape and effectiveness (Runham & Thornton, 1967; Padilla, 1985), it is important that the radula is constantly renewed during the lifetime of an individual. Radular production appears to be a dynamic equilibrium; rows of teeth are worn and shed anteriorly while they are constantly produced in the radular sac posteriorly (Runham, 1962, 1963). Therefore, an individual will replace its entire radula several times during its life, depending upon the length of the radula and its production rate.

Isarankura & Runham (1968) tested a variety of methods for marking the radulae of gastropods to determine their production rates. Among the techniques they tried, cold-shocking was the easiest, most reliable method. Cold-shocking a snail results in an anomaly in the row of teeth being produced at the time of the cold-shock. This anomaly is easy to detect and facilitates the testing of a large number of individuals. Isarankura & Runham found that the 10 gastropods species they examined were not adversely affected by the cold shock, and Padilla (unpublished data) found similar results with 8 species of patellogastropods. Therefore, we used cold-shocking to determine the rate of production of new rows of radular teeth in two species of gastropods, *Lacuna vincta* and *L. variegata*, which co-occur throughout much of their range. These two species are abundant herbivores in low intertidal and shallow subtidal communities in the Eastern Pacific. They graze a variety of macroalgae and the microalgae fouling the surfaces of sea grasses (Padilla, unpublished data). Like other members of the Littorinidae, *Lacuna* can be very abundant, but occur lower on the shore than many other littorines. In addition, they are found on macroalgae and sea grasses, and are not found grazing on rock surfaces, as is common in *Littorina*.

MATERIAL AND METHODS

All experiments were conducted at the Friday Harbor Laboratories, Friday Harbor, Washington, U.S.A. We examined two closely related species of snail, *Lacuna vincta* (Montagu) and *L. variegata* Carpenter, which were collected from shallow subtidal eel grass beds and macroalgal beds on the east shore (Brown Island) and west shore (False Bay) of San Juan Island, Washington, near the Friday Harbor Laboratories.

We examined both males and females of each species over a wide size range. *Lacuna vincta* ranged in shell length from 3.46 to 9.62 mm, while individuals of *L. variegata* ranged from 3.20 to 8.63 mm. All experimental snails were kept in flow-thought seawater tanks at ambient seawater temperature (9–11°C) until used.

We used a modification of the methods developed by Isarankura & Runham (1968) to mark the radular ribbons in experimental animals to determine radular production rates. Animals were cold-shocked, which produces a constriction and often an anomalous row of teeth on the radular ribbon. To determine the most effective method of cold-shocking, we tested animals held in seawater at −1°C for 48 hrs, −2°C for 48 hrs, and −2°C for 60 hrs. Our initial trials determined that the highest proportion of animals with detectable marks and greatest survivorship was produced at −1°C for 48 hrs, therefore these conditions were used to mark all subsequent animals. Groups of 25–50 snails were cold-shocked by being placed in −1°C seawater in an incubator maintained at −1°C (±0.5°C) for 48 hrs. After the cold-shocking period, animals were returned to ambient seawater (approximately 10–11°C) and given macroalgae (usually *Ulva*) to graze. Animals were then sacrificed at different time periods after removal from the cold-shocking: at approximately 5 days, 10 days, 15 days, and 18 days. The exact number of hours since the shocking was recorded for each individual, so that a production rate could be determined in terms of tooth rows per hour. Animals were immediately frozen to stop radular production, and stored frozen until they could be dissected.

For dissection, whole snails were placed in a 90°C water bath for a minimum of 5 minutes. Before dissection, we measured the length of the shell (the maximum distance from the apex to the base of the aperture), and then gently crushed the shell to remove the soft body. We sexed each snail (males have a penis) and then removed the radula from the soft tissue. We cleaned the radulae by sonication in a mild solution of commercial Chlorox (2–3 drops in 25 ml distilled water). We gently sonicated the radulae without heat for 1 minute, and then rinsed them with distilled water. If all of the tissue was not cleaned from the radula, it was resonicated until clean. The radulae were stored in distilled water in a refrigerator for not more than 72 hours before mounting. We mounted the cleaned radulae with a water soluble mounting medium (polyvinyl lactophenol) and observed them at 400x with a phase contrast compound microscope and a green filter to enhance contrast.

For each snail of each species we recorded: (1) shell length (mm), (2) sex, (3) the total number of tooth rows in the radula, (4) the number of hours from the end of the cold-shock until the animal was sacrificed, and (5) the number of tooth rows from posterior (youngest) end of the radula to the anomaly created by the cold-shock.

RESULTS

Final usable sample sizes were 125 for *L. vincta* (51 males and 74 females), and 134 for *L. variegata* (21 males and 113 females). The success rate in detecting anomalies was high for both *L. vincta* and *L. variegata*; when shocked at −1°C for 48 hrs, 83% of *L. vincta* had observable anomalies, and 87% of *L. variegata* had anomalies. Tooth row production rate was calculated by dividing the number of tooth rows posterior to the anomaly by the number of hours between the end of the cold-shock and death for each animal. The total number of days to replace the radula was calculated by dividing the length (in terms of tooth rows) of the radula by the production rate.

Reduced Major Axis (RMA) regression is recommend for testing for linear relationships between two variables in cases where (1) the researcher is not in control of the levels of the independent variable, (2) there is a measurement error in both the independent and dependent variable, and (3) allometric or functional relationships are being determined (Ricker, 1973; Laws & Archie, 1981; McArdle, 1988; Jolicoeur, 1990; Niklas, 1994). Therefore, RMA regression was used. We tested for relationships between: (1) total number of hours from the end of the cold-shock to death and tooth row production rate, (2) total number of rows of teeth in the radula and production rate, (3) number of days to replace the entire radula and shell length, (4) rate of tooth row production and shell length, and (5) total number of rows of teeth and shell length. Differences in slopes were tested using equations in McArdle (1988). For *L. variegata*, there was no difference between males and females for any of the above, and therefore males and females were pooled. For *L. vincta*, males and females were not different for measures (1), (2), and (4), and were therefore pooled. However, males and females differed significantly for measures (3) and (5), and were analyzed

Table 1.—Summary of RMA regression analyses. When males and females did not differ, they were pooled within species.

	Dependent Variable	Independent Variable	r^2	Slope	P Value	N
L. variegata (all)	Production rate	Hours after cold-shock	0.131		0.132	134
L. variegata (all)	Production rate	Total number Tooth rows	0.018		0.123	134
L. variegata (all)	Days to replace radula	Shell length	0.546	2.888	0.000	134
L. variegata (all)	Production rate	Shell length	0.389	−0.207	0.000	134
L. variegata (all)	Total number of rows	Shell length	0.261	5.679	0.000	134
L. vincta (all)	Production rate	Hours after cold-shock	0.002		0.588	125
L. vincta (all)	Production rate	Total number tooth rows	0.001		0.794	125
L. vincta Males	Days to replace radula	Shell length	0.179		0.210	51
L. vincta Females	Days to replace radula	Shell length	0.287	2.694	0.000	74
L. vincta (all)	Production rate	Shell length	0.222	−0.212	0.000	125
L. vincta Males	Total number of rows	Shell length	0.007		0.572	51
L. vincta Females	Total number of rows	Shell length	0.078	6.425	0.016	74

separately. Table 1 summarizes the results of the regression analyses.

For *L. variegata*, we found no significant correlation between length of time between the cold-shock and death and tooth row production rate. Therefore, it appears that the cold shock did not significantly affect production rate. We also did not find a significant relationship between production rate and total number of rows in the radula. We did find significant relationships between shell length and the total number of tooth rows, rate of production of tooth rows, and the number of days required to replace the entire radula. The total number of rows of teeth in the radula and the time required to replace the radula increased with shell length, but tooth row production rate decreased with shell length.

For *L. vincta*, we also did not find a significant correlation between length of time between the cold-shock and death and tooth row production rate or between production rate and total number of rows in the radula. As for *L. variegata*, we found significant relationships between shell length and the total number of tooth rows, rate of production of tooth rows, and the number of days required to replace the entire radula for females, but not for males. For females, the total number of rows of teeth in the radula and therefore the time required to replace the entire radula increased with shell length. However, tooth row production rate decreased with shell length.

Males and females of *L. variegata* did not differ for most of the factors assessed (Table 2). using a four-way analysis of variance (Table 3), the only difference we found between males and females were in the rate of tooth row production (males = 3.28 rows/hr, females = 2.91 rows/hr, P = 0.001), and the interaction between the total number of rows of teeth in the radula and the production rate (P = 0.002). We found little difference between the mean response of male and female *L. vincta* as well. The only significant difference found was in the interaction between the total number of rows of teeth in the radula and the production rate (P = 0.004).

The two species, *L. variegata* and *L. vincta* did not differ for most factors considered (Tables 2 and 3). The only significant differ-

Table 2. Summary of date from all experimental animals, separated by species and by sex. STD = standard deviation. N = sample size.

	Shell length (mm)	Radula length (rows)	Time after cold-shock (hours)	Rate of production (rows/hour)	Replacement of radula (days)
L. vincta – all					
Mean	5.69	69.0	271.9	2.95	23.5
Range	3.46–9.62	47–94		2.26–3 46	14.96–35.44
STD	1.21	8.1	112.2	0.26	3.5
N	125	125	125	125	125
L. vincta – males					
Mean	5.11	68.8	276.4	3.03	22.9
STD	0.76	7.5	112.2	0.28	3.3
N	51	51	51	51	51
L. vincta – females					
Mean	6.09	69.2	268.8	2.91	23.7
STD	1.32	8.5	112.8	0.23	3.6
N	74	74	74	74	74
L. variegata – all					
Mean	5.47	77.79	275.1	2.97	26.6
Range	2.74–8.63	53–99		1.99–3.70	17.43–39.69
STD	1.62	9.18	106.2	0.33	4.7
N	134	134	134	134	134
L. variegata – males					
Mean	4.37	81.24	271.6	3.28	24.9
STD	1.06	8.27	88.8	0.26	3.3
N	21	21	21	21	21
L. variegata – females					
Mean	5.68	77.15	275.7	2.91	26.9
STD	1.62	9.23	109.4	0.31	4.8
N	113	113	113	113	113

Table 3. Results of four-way analysis of variance test for differences between species and between sexes within each species. * indicates two-way interaction terms. Due to a lack of degrees of freedom, not all two-way and no three-way interactions could be tested.

Variable	Males *vs* Females *L. variegate* P value	Males *vs* Females *L. vincta* P value	*L. variegata vs L. vincta* P value
Shell length (mm)	0.427	0.230	0.159
Total number of rows in radula	0.451	0.168	0.248
Production rate (rows/hr)	0.001	0.254	0.245
Days to replace radula	0.271	0.717	0.235
Total number of rows* shell length	0.129	0.346	0.069
Production rate* shell length	0.331	0.243	0.033
Days to replace radula* shell length	0.178	0.354	0.198
Total number of rows* production rate	0.002	0.004	0.314
Total number of rows* days to replace radula.	0.378	0.814	0.535

ence we found between these two species was in the interaction between tooth row production rate and shell length (P = 0.033). For both species, tooth row production rate was around 3 rows/day (2.94 rows/day for *L. vincta*, and 2.97 rows/day for *L. variegata*). The length of the radula ranged from 47 to 94 rows (2.57 to 5.68 mm) in *L. vincta*, and 53 to 99 rows (2.80 to 7.14 mm) in *L. variegata*. The total time required to replace the radula varied from 14.96 to 35.44 days in *L. vincta*, and 17.43 to 39.69 days in *L. variegata*.

DISCUSSION

At present we have very few examples of how rapidly teeth are being produced in gastropods or other molluscs (Carriker, 1943; Isarankura & Runham, 1968). Teeth are made at the same rate as they are shed, so that the length of the radula of an individual is relatively constant (Runham, 1963). Therefore, if we know the production rate and the length of the radula, we can determine how long it takes to replace the entire radula. Isarankura & Runham (1968) found that production rate was very rapid in newly hatched individuals, and was followed by a steady decrease in production rate with age/size, and was slowest in very large animals. Our results were similar. For both species we found a negative correlation between shell length and production rate. In *L. variegata* shell length explained 39% of the variance in production rate, but explained only 22% of the variance in *L. vincta*. Isarankura & Runham (1968) also found that production rate was temperature dependent. *Littorina littorea* had a tooth row production rate of 1.77 rows per day at 10°C and 5.06 rows per day at 20°C. We did not test for such temperature dependence in *Lacuna*, as our goal was to understand production rate under natural conditions. As these animals live in the low intertidal to shallow subtidal zone in the Northeastern Pacific, they experience very little temperature fluctuation during the year, only about 2 to 3°C. It is interesting, however, that the production rate in *Lacuna* was faster than that of *Littorina*, when measured at the same temperature, approximately 10°C, and was in the middle of the range for 15 species Isarankura & Runham examined at 20°C, whose production rate ranged from 1 to 6.5 rows per day.

The radular production rate in both species of *Lacuna* was approximately 3 rows per day, and there was little inter-individual variation. However, the length of the radular ribbon varied widely among individuals, by more than a factor of two. The length of the ribbon was correlated with shell length in *L. variegata* and female (but not male) *L. vincta*, but shell length only accounted for 26% of the variance in *L. variegata*, and less than 8% of the variance in *L. vincta*. There was no correlation between the length of the radula and its production rate. Because the time required to replace the entire radula is a function of the production rate and the length of the ribbon, this was also quite variable and ranged from around 2 weeks to almost 6 weeks. As animals were from a common environment, it is unlikely that different feeding histories are responsible for the variation observed among individuals. It is possible that age could explain a lot of the variation observed. Age is only loosely correlated with size in *Lacuna* greater than 3 mm in shell length (Padilla, unpublished data). Also, *Lacuna* are sexually dimorphic, with males smaller than females. However, we did not find statistically significant differences in production rate or length of the ribbon between males and females for *L. variegata*, and for *L. vincta* the means were not different for males and females, but the males did not show a positive correlation between total number of tooth rows in the radula and shell length. Examining snails of known ages and feeding histories would allow us to address this question.

Although the radula is a key feature of gastropods, we know surprising little about the dynamics of the radula. The length of time required to replace the radula will be important for understanding the effectiveness of the radula as a tool for feeding, as worn teeth tend to be less effective than new teeth (Padilla, 1987). To determine if our results are general across other species, we need similar data on more species. In particular, we need more information on the variation among individuals. Our measured production rates are similar to those of related species (Isarankura & Runham, 1968), however, there are no other data on differences between males and females for comparison. Surprising to us was the large difference among individuals in the length of the radular ribbon. Others have looked for correlations between diet and length of the ribbon (Underwood, 1984), assuming that if teeth wear down faster they would have to be replaced at a higher rate, requiring a longer ribbon. If radular teeth are energetically expensive to manufacture, we might expect that the length of the radular ribbon should just balance the time required to make a useable tooth and the time it takes for the tooth to become too dull to be effective at feeding. Understanding the causes of this type of variability may facilitate our understanding of the ecology and evolution of complex traits such as the radula.

ACKNOWLEDGEMENTS

We would like to thank Heather Hill and John Hutchinson for helping with counting the rows of teeth. Ned Fetcher provided useful discussion and equations necessary for testing slopes of RMA regression analysis. We would like to thank the Director, staff and library of the Friday Harbor Laboratories, Friday Harbor, Washington, for space and facilities. This research was supported by grants from WARF, and NSF grants BSR-9009070, an REU supplement to BSR-9009070 and IBN-9317293 to DKP.

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