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Automatic recording of the radular activity of dogwhelks (*Nucella lapillus*) drilling mussels (*Mytilus edulis*)

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The radular activity of dogwhelks, *Nucella lapillus*, drilling mussels, *Mytilus edulis*, was monitored by piezoelectric transducers and recorded by an automatic digital system. In this way, for the first time, the drilling behaviour of dogwhelks was analysed in detail. Radular activity was similar to that previously recorded for limpets, each radular stroke (rasp) being formed by a sequence of 1–8 unit events, each probably corresponding to the application of one row of radular teeth on the substratum. During drilling, radular rasping followed a consistent pattern, each bout of radular rasping (lasting about 6–8 min) being followed by a period of radular inactivity (lasting about 35 min), when chemical dissolution is probably applied. The periodicity with which rasping was applied decreased during the drilling sequence (from about 25 min in the first quarter of the drilling process to about 40 min in the last quarter), together with a decrease in the rasping rate, i.e. number of radular rasps per min (from about 12 to about five rasps per min). Accordingly, the total number of radular rasps recorded during the first quarter of the drilling time was higher than during the other quarters (from about 3000 to about 500 rasps). The role of the radula in shell penetration was therefore rather extensive, accounting, during the first quarter of the process, for about 34% of drilling time. The results are discussed in relation to studies of chemical dissolution and mussel shell ultrastructure.

INTRODUCTION

A distinctive characteristic of muricid and naticid gastropods is the capacity to drill the calcareous shell of their prey. Early speculations (reviewed in Fretter & Graham, 1962) debated whether drilling involved (a) only mechanical scraping by the radula, (b) only chemical dissolution by secretion of the accessory boring organ, or (c) both methods. Ultrastructural analysis of bored shells has indicated that penetration is accomplished by alternating chemical dissolution and radular rasping (review in Carriker, 1981). Despite these advances, many functional and behavioural aspects of the drilling process are not fully understood. Although Carriker & Van Zandt (1972) provided a first consistent evidence of the alternated chemical and mechanical phases of drilling in *Urosalpinx cinerea*, it remains unclear whether this is a general pattern among muricids. These authors described a few predatory attacks on oysters (*Crassostrea virginica*) using microhydrophones to record the sound of radula scraping above the prey's shell. They found that long periods (about 25–30 min) of chemical attack are followed by short periods (about 1 min) of rasping. Recently, the technique for monitoring radular activity has been improved to allow automatic long-term recording of grazing by chitons and limpets (Parpagnoli & Chelazzi, 1995). In the present study, this technique has been applied to describe the drilling activity of the intertidal muricid *Nucella lapillus* on mussel shells.

Nucella lapillus drills through the shell of barnacles (*Semibalanus balanoides*) and mussels (*Mytilus edulis*) (Crothers, 1985). The drilling process accounts for a considerable proportion of the predation sequence, particularly for mussels, whose handling, comprising drilling and ingestion, can last several days (Hughes & Dunkin, 1984). During the past 20 years, the foraging behaviour of dogwhelks has been extensively studied (reviews in Crothers, 1985; Hughes & Burrows, 1994). However, while the anatomy and physiology of the accessory boring organ have been well documented (Chétail et al., 1968; Webb & Saleuddin, 1977; Andrews, 1991), little attention has been paid to behavioural aspects of the drilling process. The present study describes behavioural components of drilling on mussel shells using automatic recordings of radular activity, and is part of a broader investigation of the behavioural mechanisms of predation by dogwhelks.

MATERIALS AND METHODS

Adult dogwhelks, ranging from 30 to 40 mm in shell height, were collected monthly from Red Wharf Bay and Menai Bridge, on Anglesey (North Wales). Each dogwhelk was monitored for one predatory attack, after being deprived of food for 2–6 weeks. Variability in the period of food-deprivation arose from the need to run the experiment sequentially for different individuals because of the limited capacity of the apparatus (see below). However, drilling behaviour by dogwhelks does not vary significantly within this range of starvation periods (Rovero et al., 1999).

Mussels 25–30 mm in shell length were collected every two weeks from a sandy beach near Bangor (North

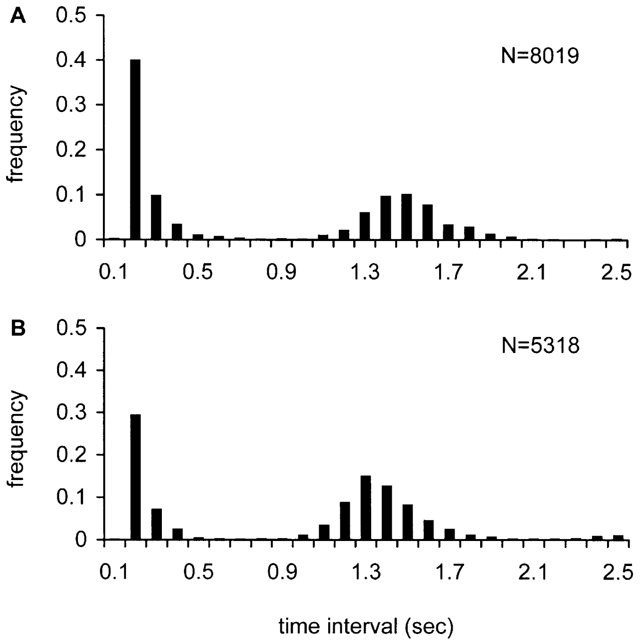

Figure 1. Frequency distribution, for a range of 2.5 s, of time intervals between successive unit events of radular rasping during two drilling processes by dogwhelks on mussel shells, (A) and (B). Each unit event probably correspond to the application of one row of radular teeth to the mussel shell. Intervals are grouped around two modes, representing respectively the time between events of the same radular rasp (0.2–0.3 s) and the time between the last event of a rasp and the first event of the following rasp (1.3–1.6 s).

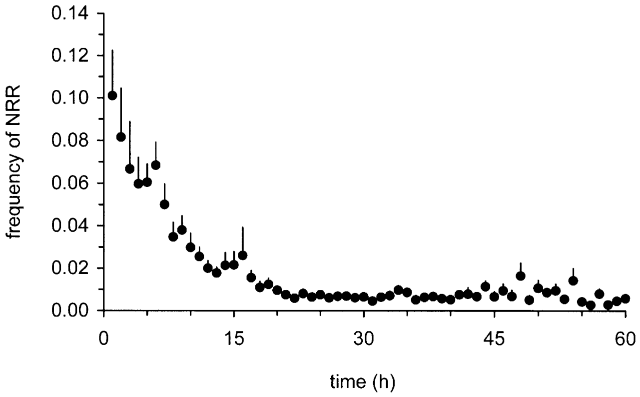

Figure 2. Frequency distribution of number of radular rasps (NRR) h^{-1} recorded for 21 dogwhelks drilling on mussel's shells. Values are plotted along a 60 h range, during which period the shell penetration process generally is accomplished. Data are means (+SE).

Wales) and maintained in aquaria on a diet of *Rhinomonas reticulata*. The size of mussels was large enough for the application of the sensors (see below) and is readily accepted by adult dogwhelks both in the laboratory (Hughes & Dunkin, 1984) and in the field (Hughes & Drewett, 1985). Experiments were conducted from January to June 1998 at 17 ±2°C under continual illumination. Aquaria contained recirculating, filtered and aerated seawater which was replaced with freshly collected seawater every two weeks.

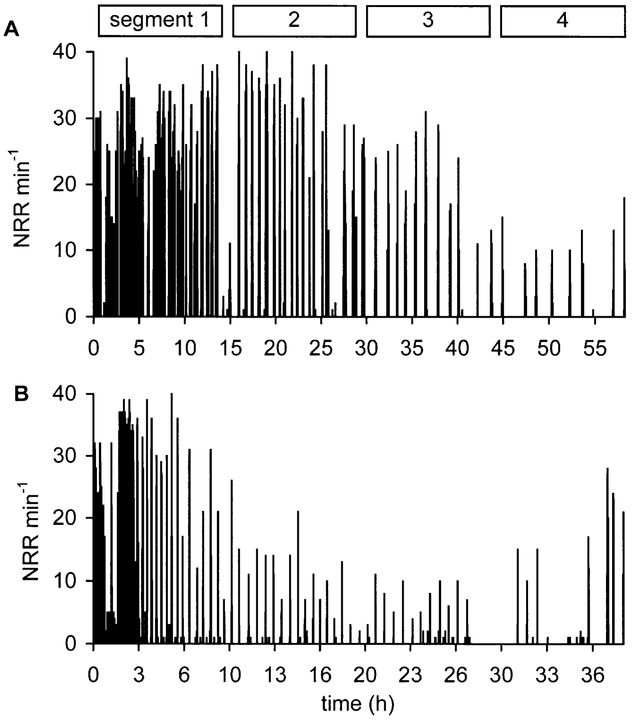

Figure 3. Time series of number of radular rasps (NRR) min^{-1}, plotted for two drilling sequences, (A) and (B), throughout the drilling process. Each sequence is divided into four segments (see text for explanation) of equal duration, as shown for the attack (A).

Experimental apparatus and procedure

The technique and data processing method for recording radular activity are described in Parpagnoli & Chelazzi (1995); here we describe only those aspects unique to monitoring drilling activity. Transducers were fixed close to the dorsal hinge of one mussel valve with cyanoacrylate glue. The other valve was glued to a PVC base. This stable and horizontal orientation of the mussel induced dogwhelks to drill the superior valve bearing the sensor, so maximizing resolution of the drilling signal. The experimental aquarium was divided by a plastic mesh into six 10×10 cm compartments, in each of which was placed a mussel bearing a sensor, together with a dogwhelk. Due to variability both in the time before each mussel was attacked by the dogwhelk and in the overall duration of the attack process, each of the six predation events was monitored during different, randomly overlapping periods of time. These events were therefore considered independent replicates. Predator and prey pairs were changed when the dogwhelk abandoned the attacked mussel.

The digital acquisition of radular activity was checked by comparison between digital and audio signals, both detected by the same apparatus. The digital recording was considered valid if the number of events stored by the system closely matched the audio signals representing strokes generated by the radula scraping the mussel shell. This comparison was done both simultaneously with data acquisition by listening to the audio signal through headphones and after having recorded the signal on a tape.

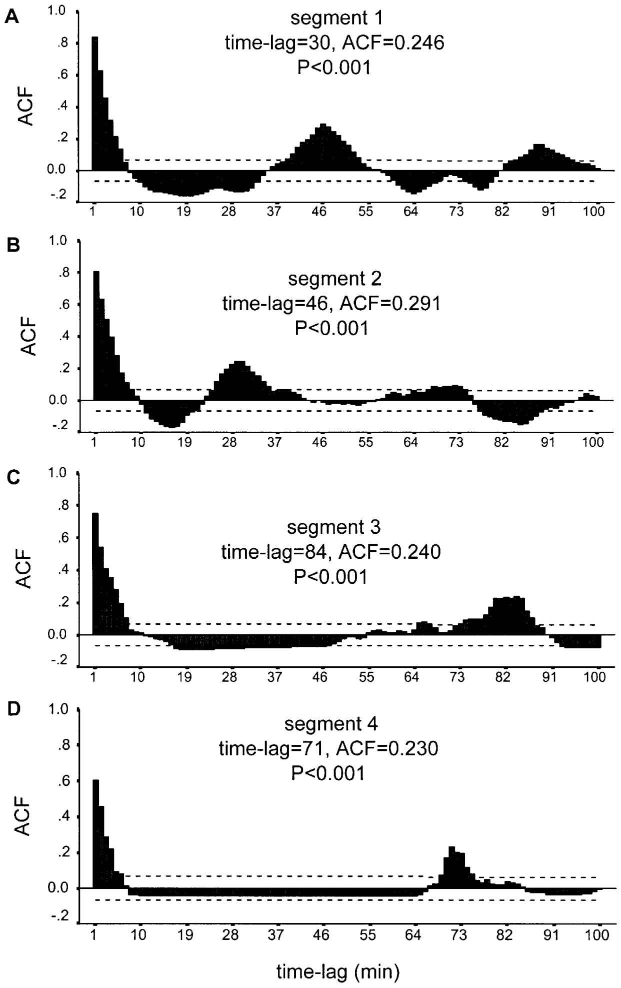

Figure 4. Values of autocorrelation coefficient (ACF) from the analysis of each temporal segment of the drilling sequence shown in Figure 3A. Horizontal dashed lines show 95% confidence limits. Time lags (min) corresponding to the higher ACF are reported in the charts, and represent the significant periodicity with which radular rasping is performed. This example shows that rasping is applied with an increasing periodicity during shell penetration (30, 46, 84 and 71 min for temporal segments 1–4, respectively).

Data were automatically stored on the hard disk of a personal computer and processed every 48 h.

Predatory events were filmed simultaneously with drilling recordings, by using a camera placed above the aquarium and connected to a time-lapse VHS video recorder that stored one frame every 3 s.

Drilling data analysis

Prey-handling behaviour of *Nucella lapillus* is composed sequentially of inspection, drilling and ingestion (Hughes & Dunkin, 1984). 'Drilling' is regarded here as the process of shell penetration whatever the mechanism involved, chemical or mechanical, whereas 'rasping' specifically refers to the radular activity, implying contact of radular teeth with the shell. Only the drilling phase of the pre-handling process was considered in the present study, drilling time (DT) being defined as the period during which the radular activity was recorded. Radular activity was not detected during either the preliminary exploratory movements of the dogwhelk on the mussel's shell or during ingestion. Integration of drilling and video data enabled the drilling phase to be distinguished from inspection and ingestion.

The original information obtained by digital recording consisted of sequences of unit events, each presumably corresponding to the application of a single row of teeth to the prey's shell. Clusters of 18 unit events, each cluster lasting 0.7–1 s and separated by gaps of 1.3–1.6 s, represented single strokes (rasps) of the radula. The recorded number of unit events per rasp depended on strength of application of the radula to the substratum (signal strength) and by recording conditions (noise level). Automatic counts of rasps per unit time were obtained by using appropriate software (Parpagnoli & Chelazzi, 1995).

To investigate the profiles of radular activity during shell penetration, the number of rasps per hour (NRR h^{-1}) was monitored throughout the drilling process. Individual frequency distributions of NRR h^{-1} were pooled to yield a profile of mean frequencies of NRR h^{-1}. Further analysis focussed on the temporal organization of rasp sequences. The number of rasps was computed on a 1-min scale, and time-series of NRR min^{-1} were drawn for each individual. Data were then normalized along the temporal-scale to accommodate variability in DT among individuals (in the range of 45–70 h, see Rovero et al., 1999 for more details). Each drilling sequence was thereby arbitrarily divided in four temporal segments of equal duration (one-quarter of DT; see Figure 3A for an example), for each of which the following parameters were computed. (a) Effective drilling time (EDT): the time characterized by occurrence of radular rasping, given by the sum of 1-min periods for which radular activity (NRR min^{-1}) was recorded. (b) Relative occurrence of radular rasping (EDT/DT): this ratio represents the fraction of drilling time characterized by radular rasping. (c) Number of radular rasps (NRR): the absolute occurrence of radular activity. The following three parameters were computed to test potential sources of variations in the NRR during shell penetration. (d) Mean number of radular rasps per minute (NRR/EDT): the instantaneous rate of drilling. (e) Periodicity: autocorrelation analysis for time-series data (Diggle, 1990) was run for each temporal segment of the drilling sequence, considering the time series of NRR min^{-1}. This procedure tested whether radular activity was applied with statistically significant periodicity, estimated for each temporal segment of drilling by the particular time-lag, if any, corresponding to the significant higher autocorrelation coefficient. (f) Duration of rasping period: computed by dividing the total length of each temporal segment by the number of rasping periods, in turn estimated by the ratio DT/periodicity. Thus the parameter is given by the formula (EDT×Periodicity)/ DT, and provides a measure of the average duration of each period of continuous radular application.

Data from each dogwhelk were combined, giving four means for each of the above parameters. Means were compared for each parameter by repeated measures ANOVA, using the Huynh–Feldt epsilon to reduce

Table 1. *Parameters of radular activity pooled for 21 drilling events by dogwhelks on mussel shells, each divided into four temporal segments of equal duration. See text for explanation of parameters.*

	Temporal segment				
Parameter	1	2	3	4	*P*
EDT (min)	243.4 ±23.7	114.0 ±12.0	100.9 ±18.0	86.7 ±10.2	<0.001
EDT/DT	0.34 ±0.02	0.17 ±0.02	0.14 ±0.02	0.13 ±0.02	<0.001
NRR	2979 ±395	726 ±146	462 ±99	500 ±123	<0.001
NRR min^{-1}	12.3 ±0.9	6.5 ±1.1	4.6 ±0.8	5.5 ±0.7	<0.001
Periodicity (min)	24.2 ±1.9	35.4 ±3.5	39.3 ±4.0	40.8 ±7.3	<0.01
Rasping period (min)	8.5 ±0.9	6.1 ±0.9	5.7 ±1.1	5.6 ±1.5	$=0.06$

Data are means ±SE; *P* is the probability that means are different across the four temporal segments, tested by repeated measures ANOVA.

degrees of freedom to accommodate for non-sphericity (Norušis, 1993).

RESULTS

Time intervals between unit events of radular scraping followed a bimodal frequency distribution (Figure 1). Intervals grouped around 0.2–0.3 s corresponded to lapses between impacts of radular teeth within a single rasp (intrarasp time), whereas those grouped around 1.3–1.6 s corresponded to intervals between the last event of a rasp and the first event of the following rasp (interrasp time). The lack of intervals between the two modes (0.7–1 s) represented the temporal criterion for separating intra- and interrasp events by the data processing system. Twenty-one attacks were analysed for radular activity and for all of them the correspondence between audio and digital signal during acquisition of data was good. Total NRR was highly variable among individuals, mostly ranging from 2000 to 4000 rasps recorded throughout the drilling process. The distribution of NRR h^{-1} is shown for a 60 h range (Figure 2), during which shell penetration was generally accomplished. Most of the radular rasping was performed in the first part of the drilling process, with higher values during the first 5 h, followed by a consistent decrease. Individual profiles of NRR min^{-1} (see examples in Figure 3) showed that drilling followed a pattern in which periods of rasping alternated with periods of total absence of radular activity. Also, rasping rhythmicity decreased progressively as the shell was drilled. Autocorrelation analysis revealed periodicity in radular activity for almost all attacks: in 81 out of 84 temporal segments analysed (i.e. four segments for 21 drilling records) a significant periodical time-lag was detected ($P<0.001$). This result is exemplified for one drilling process in Figure 4, where values of the autocorrelation coefficient for the drilling sequence of Figure 3A are plotted. The higher significant coefficients corresponded to increasing time-lags during segments 13 of shell penetration (30, 46 and 84 min, respectively), followed by a slight decrease in phase 4 (71 min).

Mean NRR recorded in segment 1 was greater than mean NRR recorded in segments 2–4, differences during shell penetration being statistically significant (repeated measures ANOVA: df=1.56, 31.28, F=47.41, $P<0.001$; Table 1). Consequently, EDT similarly decreased (repeated measures ANOVA: df=2.03, 40.74 F=48.87, $P<0.001$). If expressed as the relative contribution of radular activity to total drilling time (EDT/DT), rasping accounted for a mean of 34% DT in segment 1 to 15% in segments 24 (repeated measures ANOVA: df=2.13, 45.64, F=60.11, $P<0.001$). Also, the rate of rasping (NRR/EDT) decreased during drilling: from segment 1 to segments 2–4, mean NRR min^{-1} varied from about 12 to about six radular rasps (repeated measures ANOVA: df=3, 60, F= 27.84, $P<0.001$), with maximum values of 40 rasps min^{-1}. Periodicity of radular activity increased gradually: on average, rasping was performed each 25 min during segment 1, each 35 min during segment 2, and each 40 min during segments 3–4, the differences being significant (repeated measures ANOVA: df=2.03, 40.74, F=48.87, $P<0.001$). Mean duration of each rasping period was slightly higher for segment 1 (about 8.5 min) than segments 2–4 (about 6 min), variations being marginally non-significant (repeated measures ANOVA: df=2.73, 49.14 F=2.71, P=0.06).

DISCUSSION

The automatic technique for recording radular activity, originally designed for monitoring algal grazing by limpets on rocky substrata, satisfactorily resolved radular rasping of *Nucella lapillus* drilling mussels.

Comparison of frequency distributions of time intervals between unit event of rasping shows that radular activity in *N. lapillus*, in terms of intra- and interrasp time, and number of strokes per rasp, is almost identical to that described for limpets (Parpagnoli & Chelazzi, 1995). Thus, in *Patella caerulea*, each radular rasp is recorded as a sequence of 2–6 unit events (lasting about 0.4–0.8 s and separated by 1–3 s), 1-event rasps being due to the impact of the shell on the substratum while the limpet moves. Single rasps, on the contrary, were recorded routinely in dogwhelks, whose stable position during shell penetration prevented the occurrence of extraneous noise.

The high variability in total number of radular rasps is presumably due to variations in the thickness of the prey's shell at the borehole and/or in the drilling efficiency of dogwhelks. These aspects, as determinants of the variability in drilling time, were included in a more general investigation of prey-handling behaviour (see Rovero et al., 1999). However, it was assumed that such variables did not affect the general temporal organization of the radular activity, whose analysis is the main purpose of this study.

We assume that non-rasping periods correspond to the chemical action by the accessory boring organ (Chétail et al., 1968; Chétail & Fournié, 1969). The occurrences of radular activity and, indirectly, of chemical application, are in qualitative agreement with drilling profiles reported for *Urosalpinx cinerea* (Carriker & Van Zandt, 1972), although in that study periodicity and rate of radular rasping were not quantified, therefore significant variations in the drilling process were not detected.

The function of the radular activity during shell penetration is the removal of shell layers softened by chemical attack at the bottom of the borehole, thus rasping was considered to temporally play a minor role in shell penetration (Carriker, 1981). On the contrary, it was found that during the first quarter of the drilling process, radular rasping accounts for a consistent proportion of time (about 35%). This can be explained when considering the ultrastructure of mussel shell, whose external layer is made up of organic membranes (Kobayashi, 1969). Chemical dissolution, although important for solubilizing the organic matrix, is primarily a mechanism for dissolving the inner mineralized layers (Carriker, 1978). Probably, frequent and intense mechanical scraping is necessary for penetrating the external organic layers, after which dogwhelks progressively shift to a pattern of longer periods of chemical dissolution necessary to dissolve the crystalline layers.

Although further interdisciplinary studies would be required to verify this hypothesis, it is clearly shown here that *N. lapillus* is able to drill the mussel's shell according to a rather conservative pattern of chemo-mechanical activity, shown amongst individuals from different populations.

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