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Non-invasive techniques for studying behavioural mechanisms and physiological states of marine invertebrates

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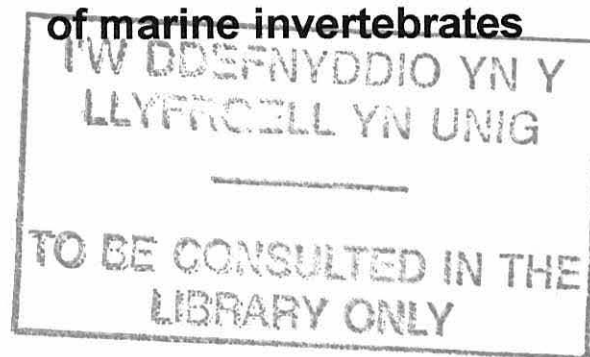
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University of Wales, Bangor

School of Biological Sciences

**Non-invasive techniques for studying behavioural
mechanisms and physiological states
of marine invertebrates**



A thesis submitted by

Francesco Rovero

(M.Sc. Natural Sciences, Florence)

in candidature for the degree of Doctor of Philosophy

April 2000



“Ogni azione fatta dalla natura è fatta nel modo minimo”

(Every action done by nature is done in the shortest way)

Leonardo da Vinci's notebooks, Florence, 1508

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Preface

This thesis presents five papers written by the candidate. All 5 papers have been published or are in press in international journals, namely *Animal Behaviour* (chapters 2, 4, 5), *Journal of the Marine Biological Association of the United Kingdom* (chapter 3) and *Journal of Animal Ecology* (chapter 6). Reviews of the paper reported in chapter 4 have appeared on *Science News*, *New Scientist* and *Nature Australia*.

Each paper deals with rather different aspects of the behavioural ecology of selected marine invertebrates (dogwhelks, mussels and crabs), but they all rely on the same experimental approach, which is the use of non-invasive techniques for monitoring underlying behavioural mechanisms and physiological states of animals. This common framework is presented in the general introduction (chapter 1), papers being then reported in the following chapters (2-6), followed by a general discussion (chapter 7). References are collected in chapter 8. Reprints and proofs are appended.

The study was carried out within the framework of the EUROROCK project (MAST III, 1996-1999), funded by the European Union.

Summary

Behavioural ecology is ultimately aimed at understanding the evolutionary significance of behavioural traits, i.e. how behaviour influences fitness. Both theoretical and experimental investigations follow an economic approach: behavioural patterns are analysed in terms of costs and benefits of alternative courses of action, assuming that natural selection would promote optimization of the balance between costs and benefits. It has been progressively recognized that the detailed knowledge of fitness consequences of behavioural acts requires the understanding of mechanisms and physiological processes underlying behavioural decisions. The present study is aimed at testing new techniques for monitoring such variables, which are based on the deployment of small transducers externally mounted on animals. This non-invasive monitoring allows the recording of behavioural patterns while minimizing associated disturbance. Transducers were applied on hard-shelled marine invertebrates: dogwhelks (*Nucella lapillus*), common mussels (*Mytilus edulis*) and shore crabs (*Carcinus maenas*), which are also ideal subjects for laboratory studies and have been already extensively studied in behavioural ecology. Papers reported in chapter 2 and 3 deal with the foraging behaviour of dogwhelks. A transducer for recording mechanical vibrations was used to directly record, for the first time, the drilling behaviour of dogwhelks penetrating mussel shells. This in turn allowed the effect of experience on handling time to be quantified. The remaining three studies are based on the use of infrared phototransducers for monitoring cardiac activity. Because of the relationship between circulatory and respiratory systems, measuring heartbeat rate provides information on the consumption of oxygen, and thus, on the energy demand associated with particular performances. In the study reported in chapter 4, this technique was used for monitoring responses of mussels to risk of attack and predation by dogwhelks. In the presence of effluent from dogwhelks, heartbeat rate of mussels significantly increased without any apparent behavioural response. Cardiac activity increased further when under attack. These responses might represent an adaptive trade off between energy budget and risk of predation. In chapter 5, transducers were used to monitor heartbeat of crabs involved in fights against conspecifics of various sizes. The relation between cardiac and respiratory rates was assessed and, consequently, the energy demand of different fighting strategies could be quantified. Costs of aggression were determined by the time spent fighting but not by the fighting strategy. Moreover, post-fighting alertness incurred metabolic costs, independent from the previous behavioural effort, which are probably adaptive against the risk of being injured and/or losing contested resources. The remaining study focussed on prey-handling behaviour of crabs. By monitoring heartbeat rate, energy costs of handling mussels of various sizes could be quantified for the first time. Surprisingly, costs represented an almost irrelevant proportion of corresponding gains. Moreover, the tendency of profitability (gross energy gain per unit of handling time) to increase with prey-size was weakened by including energy cost and thus time was judged to be a more appropriate currency for costing. Throughout the study, the mechanistic approach to behavioural analysis was essential for collecting crucial and unique information on how behaviour is related to fitness.

Acknowledgements

I am extremely thankful to Professor Roger Hughes for his invaluable, constant and friendly supervision. I am also grateful to Professor Guido Chelazzi who gave me the opportunity of doing this research project and helped me during those phases of the work that were carried out in Florence.

I would like to thank Dr. Nia Whiteley for advices on interpretation of data on heartbeat rate and for helping me during experiments on respirometry and Dr. Kei Kawai for helping me during collection and maintenance of animals. Many thanks to the referees, whose criticism and comments improved the quality of the study.

Thanks to my parents, who supported me in many ways and thanks to those friends who came to visit me in Bangor.

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Chapter 1. General introduction

“Behavioural ecology was spawned as a wayward offspring of ethology, focusing on just one of Tinbergen’s (1963) ‘four questions’ (function) and eschewing the other three (mechanism, ontogeny, evolutionary history). Inexorably behavioural ecologists are being drawn back into the study of mechanisms. A return to mechanisms does not imply turning the clock back... Behavioural ecologists, approaching mechanisms from a sophisticated and coherent functional framework, will often ask unusual questions and come up with surprising answers.” (Krebs & Davies 1991)

These considerations, from the preface of one of the most authoritative texts on behavioural ecology, epitomise the conceptual approach underlying the present study. It is useful to start by considering that much of evolutionary biology is ultimately aimed at understanding the usefulness of traits, based on the assumption that traits are “chosen” by natural selection because of their adaptiveness (Darwin 1859). In the context of behavioural ecology, the main goal therefore is to understand how behaviour influences fitness, i.e. how behaviour is related to expected survival and reproduction (Mills & Beatty 1979).

The main contemporary framework for describing, understanding and predicting behavioural patterns relies on the use of the metaphor of the animal as ‘decision-maker’ (Krebs & Kacelnik 1991). Animals can be thought of as deciding whether to behave in this way or that, for example whether to feed on patch ‘a’ or ‘b’ or choose prey ‘x’ or ‘y’. Following an evolutionary approach, fitness consequences of behavioural decisions can be inferred by analysing decisions in terms of cost and benefit of alternative courses of action, assuming that these costs and benefits affect survival and reproduction. Since natural selection eventually will tend to produce outcomes that represent the best achievable balance of costs and benefits, theoretically animals will tend to maximize benefits and minimize costs.

Such an optimality approach to the analysis of behavioural adaptations has inspired a wealth of experimental studies, which indeed have shown that behavioural patterns can be explained through the principle of maximizing gains to costs. In this context, the study of foraging behaviour has played a leading role (Stephen & Krebs 1986; Hughes 1993) probably because, in comparison with other behaviour (for example mating and fighting), the measure of a currency for benefits (food acquisition) and for costing (time spent foraging) is relatively straightforward.

However, while the theoretical analysis of the relationship between behavioural decisions and fitness has generated progressively more sophisticated and integrated optimality models (Krebs & Kacelnik 1991; Hughes 1993; Houston & McNamara 1999), it has become of pressing importance to study experimentally the mechanisms and physiological parameters included in those models, which underlie behavioural decisions. Measurement of such variables should facilitate estimation of the costs and benefits of alternative decisions, and therefore is crucial for answering central questions in behavioural ecology.

The present study tested new techniques for measuring behavioural mechanisms and physiological parameters thought to be important determinants of costing and therefore of decision-making. The techniques were based on the use of small transducers externally mounted on animals; this non-invasive monitoring allowed behavioural patterns to be recorded with minimal disturbance to the subjects. Transducers were applied to the hard-shelled marine invertebrates: dogwhelks, mussels and crabs, which are also ideal subjects for laboratory studies and have been already extensively studied in behavioural ecology (e.g. Elner & Hughes 1978; Hughes & Dunkin 1984; Petraitis 1987; Marshall & McQuaid 1993; Hughes & Burrows 1994; Huntingford et al. 1995; Sneddon et al. 1997b). Through experimental manipulation, animals were induced to perform specific behavioural responses and consequently, by comparison of experimental treatments, underlying mechanisms of alternative behavioural decisions could be monitored. The different studies that form this thesis will here be briefly introduced at the light of this common theoretical and experimental framework, whereas the literature review and research background specific to the different topics are reported in the introduction of each paper.

Papers reported in chapter 2 and 3 deal with the foraging behaviour of the dogwhelk (*Nucella lapillus* L., Mollusca, Gastropoda), a shell-boring intertidal predator. A transducer for recording mechanical vibrations, originally implemented for monitoring algal grazing by limpets (Parpagnoli & Chelazzi 1995), was used to record the drilling behaviour by dogwhelks on mussel shells. This complex behavioural mechanism, which previously had not been directly monitored, constrains the foraging strategy of dogwhelks and is therefore critically related to fitness.

The other three studies of the thesis are based on the use of infrared phototransducers for monitoring cardiac activity. In any animal species, heartbeat rate is a crucial indicator of physiological state. Because of the relationship between circulatory and respiratory systems, measuring heartbeat rate provides information on consumption of oxygen, and thus, on the energy demand associated with particular performances. The availability of a non-invasive technique for measuring such a crucial parameter has opened up a new avenue of approach for behavioural investigation. In order to explore the potentiality of application, the technique was applied to various behavioural patterns: (1) responses to perceived predation risk, (2) contests over limited resources and (3) handling prey.

The paper reported in chapter 4 again studies the dogwhelk-mussel system, but the attention is shifted from the predator to the prey. The aim is to describe responses of common mussels (*Mytilus edulis* L., Mollusca, Bivalvia) under threat of predation and under physical attack by dogwhelks. Predation risk is known to be an important factor constraining behavioural strategies (Godin 1990; Sih 1993); however, underlying physiological alterations of the energy budget associated with alertness are almost unknown.

Chapter 5 and 6 seek to estimate the energetic cost of fighting and prey-handling, respectively, in shore crabs (*Carcinus maenas* L., Crustacea, Decapoda). Simultaneous measurement of heartbeat and oxygen consumption rate of subjects kept in a respirometer enabled a correlation between cardiac and respiratory rates to be established. Using this correlation, fitness consequences of various behavioural strategies were analysed in energetic terms, which previously had been technically difficult. In chapter 5, the energetic costs of alternative fighting behaviours were estimated and found to be less significant than prolonged state of alertness after fighting. In chapter 6, the energetic cost of handling different food items could be referred to corresponding energetic values of flesh ingested. By computing benefit/cost ratios, classic optimality theories on foraging could be tested and the validity of currencies, such as time, previously used for costing, could be assessed.

Thus, the research reported in this thesis represents an attempt to integrate physiological and behavioural approaches in the study of adaptiveness of behavioural traits.

Chapter 2. Effect of experience on predatory behaviour of dogwhelks

2.1. Abstract

We used an acoustic transducer to monitor the radular activity of dogwhelks, *Nucella lapillus*, drilling mussels, *Mytilus edulis*, in the laboratory and we examined the effect of dietary experience on the prey-handling behaviour. For the first time, phases of inspection, penetration and ingestion could be distinguished directly and consequently the prey-handling process analysed in detail. Dogwhelks with different field-based experience of mussels showed different handling behaviour. Those collected from a mussel-dominated shore more readily adopted the faster method of penetration between the slightly gaping valves, instead of the slower method of drilling through the shell. Those collected from a barnacle-dominated shore took a significantly longer time before attacking the mussel and then were unable to switch from drilling to penetration through the gape between valves. Experience of specific prey in the field, by reducing handling time, could promote the fitness by reducing exposure to environmental hazards. Laboratory attempts to train dogwhelks from the barnacle-dominated shore to use the gape-penetration method failed, suggesting that functional constraints, e.g. injection of a relaxant when penetrating through the gape and/or genetically controlled behavioural traits, could limit the ability to learn handling skills.

2.2. Introduction

Food acquisition and risk avoidance are critical, but often mutually conflicting, determinants of fitness (Stephen & Krebs 1986; Sih 1993). Increasing experimental evidence supports the theoretical prediction that animals are behaviourally adapted to balance food acquisition and risk avoidance (e.g. Milinsky & Eller 1978; Lima et al. 1985; Hughes & Burrows 1991). Such constrained optimization of energy intake is accomplished in different ways, for example by reducing foraging activity (Lucas 1990; Burrows & Hughes 1991), by appropriate choice of prey, or by reducing prey handling time (Elner & Hughes 1978; Palmer 1983). Specifically, intertidal species that feed on hard-shelled prey, for example muricid gastropods and predatory crabs, face persistent handling constraints which, together with the need to avoid mortality,

influence foraging behaviour (Lawton & Hughes 1985; Brown & Richardson 1987). These species generally are able to improve handling skills through experience of prey, so shortening handling time (Cunningham & Hughes 1984; Hughes & Dunkin 1984; Micheli 1995; Serra et al. 1997). Despite theoretical importance of learned improvement in prey-handling skills (e.g. Hughes 1979), underlying mechanisms remain poorly understood, probably because of difficulties in quantifying the complex handling processes involved (Fairweather & Underwood 1983; Godin 1990; Hughes & Seed 1995). Increasing this understanding would represent an important step towards a progressively more integrated analysis of the relationship between foraging behaviour and fitness (Houston et al. 1988).

The North Atlantic muricid gastropod Nucella lapillus is an ideal subject for investigating the influence of experience on handling behaviour. Dogwhelks forage almost exclusively on barnacles and mussels by drilling the shell, using combined radular scraping and chemical dissolution (Crothers 1985). Handling times of these sessile prey can span several tidal cycles (Hughes & Drewett 1985), thus influencing exposure to environmental hazards such as dislodgement by waves (Etter 1996) and predation (Palmer 1990; Vadas et al. 1994). Experience of prey can greatly reduce handling times for barnacles (Dunkin & Hughes 1984) and mussels (Hughes & Dunkin 1984). Precisely how experience affects handling time in this way remains unknown, since drilling has never been directly recorded and is normally indistinguishable from ingestion (Bayne & Scullard 1978). Attempts to directly monitor the drilling activity of a predatory gastropod have been made only on the muricid Urosalpinx cinerea, using microhydrophones to record the noise made by the radula scraping the prey's shell (Carriker & Martin 1965; Carriker & Van Zandt 1972). Recently, this technique has been enhanced for accurately monitoring the grazing activity of herbivorous chitons and limpets (Parpagnoli & Chelazzi 1995) and in the present study it was used for continuously monitoring the radular activity of dogwhelks feeding on mussels. In this way, behavioural components of the prey-handling process could be quantified and the influence of experience on these processes examined. The study illustrates how a mechanistic approach to the analysis of behavioural mechanisms, not amenable to field monitoring, can aid investigation of the adaptive interaction between constraints (handling of prey) and state variables (level of experience).

2.3. Methods

General methods

Dogwhelks were collected prior to the beginning of each experimental session from two rocky shores on Anglesey, North Wales. The first shore, Red Wharf Bay, was densely populated by barnacles and mussels, whereas the second, Menai Bridge, was populated only by barnacles. It was assumed therefore that dogwhelks from the first population were experienced at handling both barnacles and mussels, while dogwhelks from the second population were inexperienced with mussels. Only adult dogwhelks were collected, recognizable by the presence of apertural teeth and erosion of the apertural margin that typically accompany cessation of shell growth (Crothers 1985).

Mussels 25-30 mm in shell length were collected every two weeks from a sandy shore near Bangor, North Wales. This size was considered large enough for the application of the sensors (below) and has been reported to be selected by adult dogwhelks both in the laboratory (Hughes & Dunkin 1984) and in the field (Hughes & Drewett 1985). Barnacle-covered rocks, about 10 cm in diameter, were used for maintaining the control batches of dogwhelks and were collected from the same site as the mussels. Prey were fed continually with *Rhinomonas reticulata*. Except for the mussels offered to the dogwhelks as prey, all individuals used in the experiments were eventually released at the collection sites.

Experiments were conducted from January to June 1998 at 17 ± 2 °C under continual illumination. The experimental aquaria contained recirculating, aerated, filtered sea water that was changed every two weeks.

Video and drilling recording

A camera placed above the experimental aquarium was connected to a time-lapse video-recorder, capturing frames at 3 sec intervals. A piezoelectric transducer was fixed with cyanoacrylate adhesive to one of the valves of the mussel, close to the dorsal hinge. The opposite valve was glued to a PVC base, forcing dogwhelks to drill the upper valve bearing the sensor, so maximizing the resolution of the signal. The stable, horizontal position of the mussel facilitated video-monitoring of behavioural patterns. Correct digital encoding of radular activity was checked for each predatory

event by comparing digital with audio signals that were monitored either by tape recording and/or by listening through headphones. When audio signals, forming a sequence of pulses as the radular teeth scraped over the mussel shell, corresponded to an equal number of digitally encoded events, the recording was considered valid. We discarded recordings of any predatory event accompanied by extraneous noise or by signals of low resolution. Data were stored automatically on the hard disk of a Personal Computer and files exported every 48 h for processing (Parpagnoli & Chelazzi 1995), yielding the number of radular rasps performed by each dogwhelk, both in a given interval of time and throughout the predatory sequence.

Experimental protocol

The experimental aquarium was divided by plastic mesh into six 10x10 cm compartments, representing 3 experimental and 3 control treatments. In each compartment was placed a dogwhelk of known shell height and a mussel of known shell length, bearing a transducer. Predator/prey pairs were replaced once a dogwhelk had abandoned the mussel, having completed its attack. The mussel was inspected for position of the borehole among quadrants of the shell (see Hughes & Dunkin 1984 and diagram in Table 1) and for the presence of partial holes or any other drilling mark, using a 40x dissection microscope. Shell thickness at the borehole was measured with a vernier micrometer. Observed frequencies of drilling-site location were compared with those expected from non-selective drilling, i.e. equal number of bored holes per quadrant, using chi-square tests (Siegel & Castellan 1989). Observed distributions of drilling-site location were compared between mussel-experienced and mussel-inexperienced dogwhelks, again using chi-square tests.

Attacks were classified in two categories according to whether mussels were penetrated by drilling a hole through the shell (bored hole method) or by insertion between slightly gaping valves (gape insertion method). Inspection of the empty mussel valves, supplemented by video analysis (see below), allowed unambiguous assignment of attacks to one of the two categories. Cases of hole drilling at the edge of the valve were assigned to the gape insertion method. Differences in the relative occurrence of attack methods between experimental and control batches were compared by chi-square tests.

Experiment 1: effect of field-based experience

To test whether a long-term diet history affects handling times, the foraging behaviour of dogwhelks from Red Wharf Bay (mussel-experienced) and Menai Bridge (mussel-inexperienced) was compared. Dogwhelks were first deprived of food for 4 weeks, to standardize hunger levels. The experiment then ran for 10 weeks, until at least 20 attacks per treatment had been monitored.

Experiment 2: effect of laboratory-based experience

Dogwhelks from the Menai Bridge population, inexperienced with mussels, were divided in two batches and maintained for 9 weeks in aquaria with mussels (experimental batch) or barnacles (control batch), respectively. Dogwhelks maintained with mussels had eaten an average of 9 mussels by the end of dietary training and thus were assumed to have become experienced in handling mussels. Following dietary training, dogwhelks were deprived of food for 2 weeks to standardize hunger levels. This relatively short period of food deprivation was used in order to minimize attenuation of learned prey-handling skills.

Experiment 3 : attack methods on aggregated mussels

This experiment was performed to test whether the occurrence of attack methods in experiment 1 and 2 could have been biased by the isolation and unnatural orientation of mussels cemented to the substratum. Two batches of 50 dogwhelks from Menai Bridge and Red Wharf Bay populations (mussel-inexperienced and mussel-experienced, respectively) were deprived of food for 2 weeks, and then maintained in aquaria with 25-30 mm mussels that had been allowed to aggregate into clusters of 20-30 individuals, until a minimum of 100 attacks per batch had been recorded. Mussels were inspected regularly and empty shells were classified for attack method and drilling-site selection. The experiment lasted 9 weeks.

Assessment of parameters of foraging behaviour

Prey-handling behaviour of *N. lapillus* consists sequentially of inspection, drilling and ingestion (Hughes & Dunkin 1984). Video and drilling recording produced complementary information that, once integrated, enabled temporal quantification of the three prey-handling phases (Fig. 1).

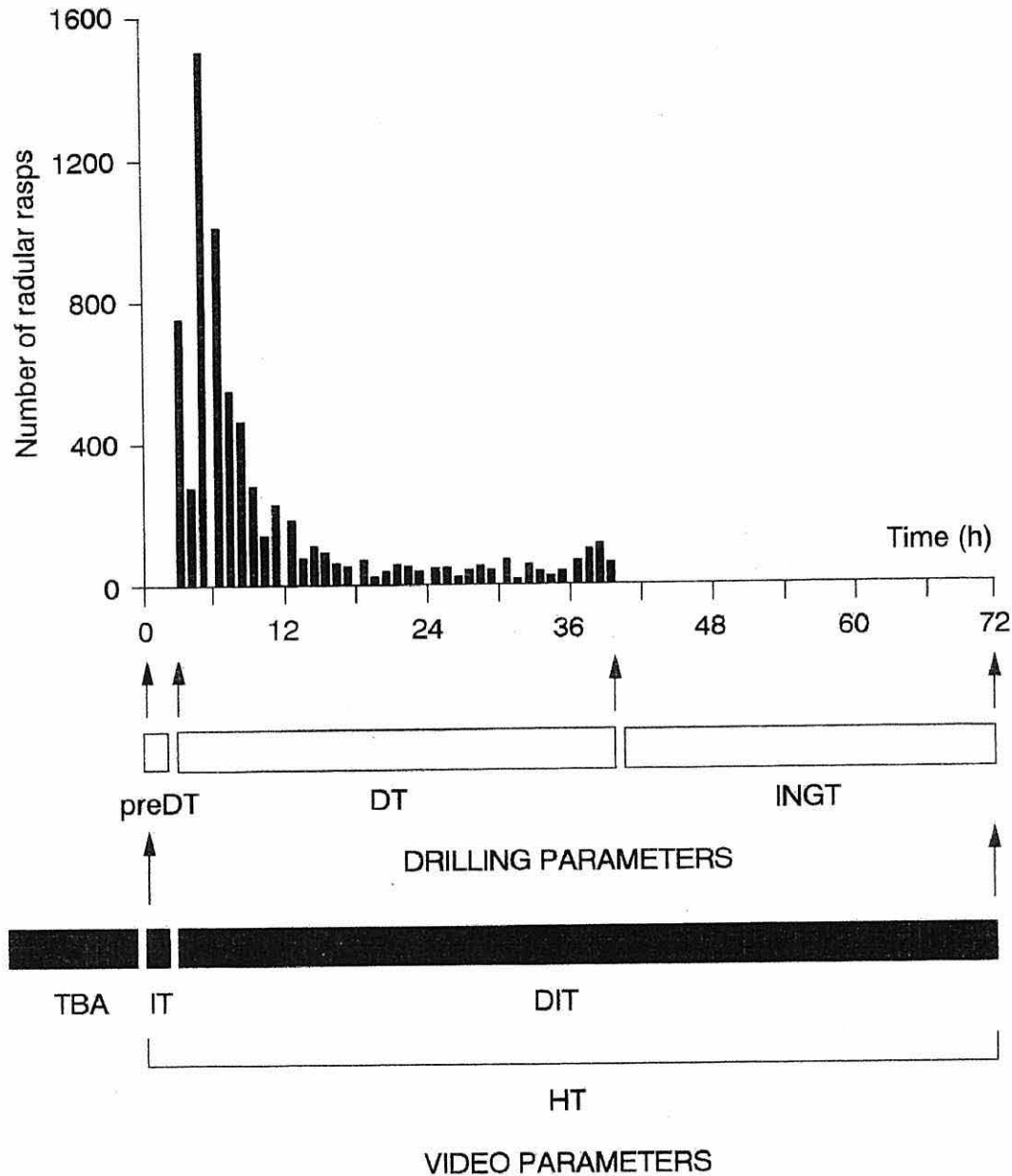


Figure 1. Diagram of criteria used for assessing components of predatory behaviour, based on integrated drilling and video data. Drilling time (DT) is computed as the period characterized by radular activity, estimated by the number of radular rasps. Pre-drilling time (preDT) and ingestion time (INGT) are computed as the periods before and after drilling time, respectively. From the video parameters, total handling time (HT) is partitioned into inspection (IT) and drilling and ingestion time (DIT), which themselves cannot be separated. Time before attack (TBA) is computed as the period elapsing from placement of the dogwhelk in the foraging compartment to the start of the attack.

Video analysis

Each attack was analysed from the moment the dogwhelk was placed together with the mussel to the moment it abandoned the prey. Prey-handling phases were measured

in minutes and then converted to hours using two decimal places. The period elapsing until the mussel was contacted was defined as time before attack; the phase of exploratory movements of the dogwhelk on the shell of the mussel before adoption of a consistent drilling position, was considered as inspection time. If the prey was attacked, a phase of indistinguishable drilling and ingestion was quantified. Total handling time was therefore given by inspection time + drilling and ingestion time.

Any other movements of dogwhelk or mussel were recorded. In particular, cessation of valve gaping or foot extension by the mussel was used together with drilling-analysis data (below) for estimating the beginning of ingestion.

Drilling analysis

Within a predatory sequence, the period characterized by occurrence of radular activity was defined as drilling time. It was expressed also as a fraction of total handling time (drilling time / handling time). Any interval since the beginning of inspection to the beginning of drilling was defined as pre-drilling time, whereas the phase that followed drilling time was defined as ingestion time.

Drilling activity was quantified by scoring the total number of radular rasps (Fig. 1). An index of the intensity of drilling was expressed as the number of radular rasps per hour, given by the ratio number of radular rasps / drilling time. Unless specified, values are given as means \pm SE.

Foraging parameters of experimental and control dogwhelks and differences between attack methods within batches were compared using the two-tailed Mann-Whitney - Wilcoxon test for unpaired data (Siegel & Castellan 1989). Analysis of covariance (Sokal & Rohlf 1995) was used to examine potential effects of dogwhelk size, mussel size, and starvation period on handling time. Pearson correlation analysis was used to examine relationships between the above covariates and behavioural measures (Sokal & Rohlf 1995).

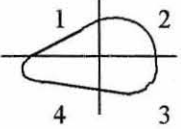
2.4 Results

Attack methods and variation of total handling time with experience

In experiment 1, the gape insertion method was used significantly more frequently by dogwhelks from Red Wharf Bay, which had gained experience of mussels in the field,

than those from Menai Bridge, which had not (Table 1, chi-square test: $\chi^2_1 = 8.92$, $P < 0.005$). For Red Wharf Bay dogwhelks, handling time was 53% shorter for the gape insertion than for the bored hole attack method (Table 2, Fig. 2, Mann-Whitney - Wilcoxon test: $W = 788.0$, $N_1 = 17$, $N_2 = 26$, $P < 0.0001$). For Menai Bridge dogwhelks handling time was 48% shorter for the gape insertion method, but this difference was not tested statistically because of the small number of gape insertion attacks recorded.

Table 1. Number of predation events per attack method and number of bored hole attacks per drilling site on the shell of the mussel (see bottom left diagram), for mussel-experienced (E) and mussel-inexperienced (I) dogwhelks

		Experiment 1		Experiment 2		Experiment 3	
		E	I	E	I	E	I
Attack method	hole	26	30	19	25	79	119
	gape	17	3	5	2	50	13
Drilling site	1	12	9	9	9	39	49
	2	10	10	8	9	30	42
	3	1	5	2	3	6	13
	4	4	6	1	3	4	15

hole = penetration through a drilled hole; gape = penetration between the gaping valves.

Table 2. Video parameters per attack method for mussel-experienced (E) and mussel-inexperienced (I) dogwhelks

	Batch	Method	N	TBA (h)	IT (h)	HT(h)
Experiment 1	E	hole	26		2.35 ± 0.43	106.8 ± 5.25
		gape	17		1.40 ± 0.73	49.7 ± 3.68
		total	43	4.04 ± 1.03		
	I	hole	30		1.35 ± 0.24	100.51 ± 4.18
		gape	3		1.33 ± 1.22	51.53 ± 7.69
		total	33	13.35 ± 2.75		
Experiment 2	E	hole	19		1.03 ± 0.33	89.08 ± 4.03
		gape	5		0.08 ± 0.07	36.52 ± 2.96
		total	24	20.09 ± 4.26		
	I	hole	25		0.54 ± 0.12	99.67 ± 6.85
		gape	2		0	48.62 ± 6.29
		total	27	21.47 ± 7.19		

Values are means \pm SE. TBA = time before attack; IT = inspection time; HT = handling time; hole = penetration through a drilled hole; gape = penetration between the gaping valves.

In experiment 2, the gape insertion method was used too infrequently for statistical analysis (Table 1). With naturally aggregated mussels (experiment 3), gape insertion attacks were used significantly more frequently by experienced than by inexperienced dogwhelks (Table 1, chi-square test: $\chi^2_1 = 29.78$, $P < 0.001$). Relative frequencies of gape insertion and bored hole attack methods used were not significantly different between experiment 1, when mussels were isolated, and experiment 3, when they were aggregated (Table 1, chi-square test: $\chi^2_1 = 0.008$, $P = 0.92$).

As expected from the small range of prey sizes used, handling time was not significantly correlated with mussel size. Handling time was significantly correlated with dogwhelk size only for mussel-experienced dogwhelks using the bored hole attack method in experiment 1 (Pearson correlation test: $r = -0.42$, $N = 26$, $P < 0.03$). Also in experiment 1, dogwhelks using the gape insertion attack method were significantly larger than those using the bored hole method (Fig. 2, Mann-Whitney - Wilcoxon test: $W = 455.0$, $N_1 = 17$, $N_2 = 26$, $P < 0.05$).

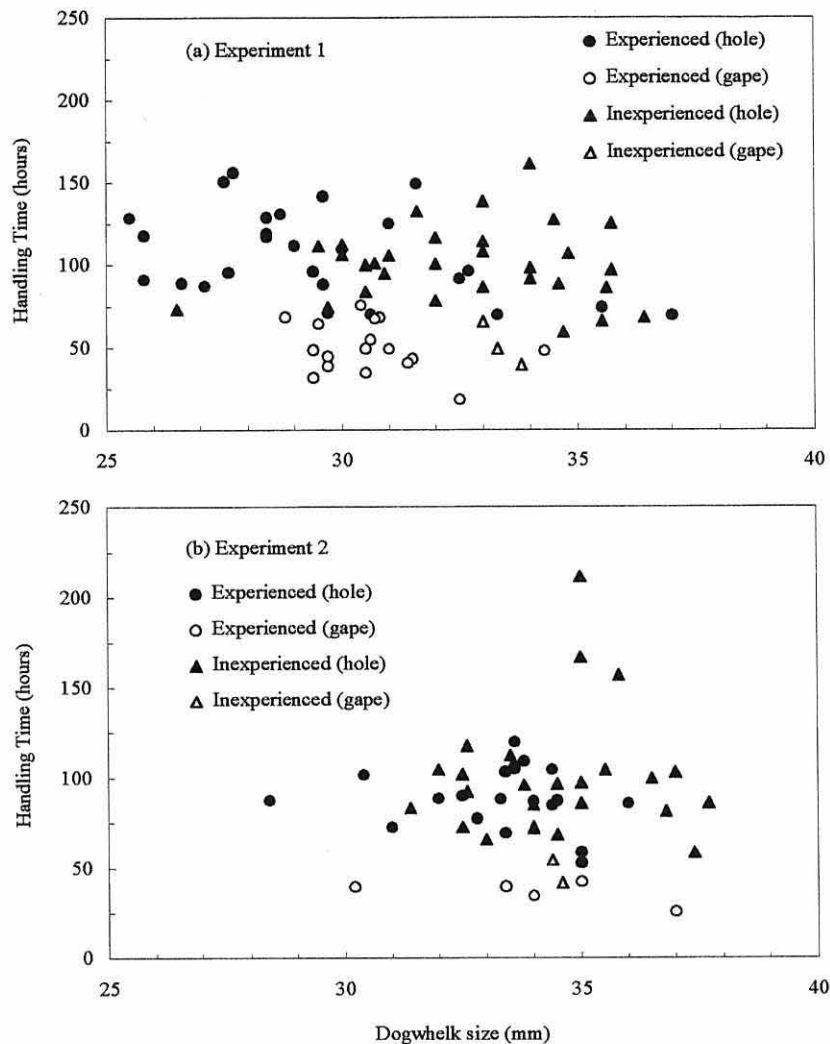


Figure 2. Handling time (h) plotted against dogwhelk size (mm in shell length) for experiment 1 (a) and experiment 2 (b), respectively. Values are categorized by experience of the dogwhelk, i.e. previously having attacked mussels (mussel-experienced) or having attacked only barnacles (mussel-inexperienced), and by attack method used, i.e. drilling a hole through the shell (hole) or penetration between the slightly gaping valves (gape).

In experiment 2, as in experiment 1, handling time for the gape insertion method (52 ± 2.96 h) was significantly shorter than for the bored hole method (89.08 ± 4.03 h) (Table 2, Mann-Whitney - Wilcoxon test: $W = 285.0$, $N_1 = 5$, $N_2 = 19$, $P < 0.001$). This comparison is based only on dogwhelks experienced with mussels, since too few mussel-inexperienced dogwhelks used the gape insertion attack method.

Although, in experiment 1, dogwhelks from Menai Bridge were larger than those from Red Wharf Bay, this difference did not significantly confound comparisons of handling time (experiment 1, ANCOVA: $F_{51,1} = 2.40$, $P = 0.128$). Experience had no significant effect on handling time for the bored hole attack method (Fig. 2, Mann-

Whitney - Wilcoxon test: experiment 1: $W = 791.0$, $N_1 = 26$, $N_2 = 30$, $P = 0.41$; experiment 2: $W = 578.0$, $N_1 = 19$, $N_2 = 25$, $P = 0.72$).

Drilling site selection

Drilling sites on the shell of the mussel were significantly non-randomly distributed for dogwhelks experienced with mussel in experiment 1 (chi-square test: $\chi^2_3 = 15.83$, $P < 0.01$) and for both mussel-experienced and mussel-inexperienced batches in experiment 3 (chi-square test: experienced: $\chi^2_3 = 41.05$, $P < 0.001$; inexperienced, $\chi^2_3 = 27.54$, $P < 0.001$). Quadrants 1 and 2, on the hinge side of the valve, were selected more frequently for drilling than quadrants 3 and 4, but there was no significant difference in usage of these quadrants by mussel-experienced and mussel-inexperienced dogwhelks (Table 1).

Variation of foraging parameters with experience

In experiment 1, dogwhelks inexperienced with mussels took significantly longer than mussel-experienced individual to attack each mussel (Table 2, time before attack inexperienced: 13.35 ± 2.75 h, experienced: 4.04 ± 1.03 h, Mann-Whitney - Wilcoxon test: $W = 1026$, $N_1 = 33$, $N_2 = 43$, $P < 0.005$), whereas in experiment 2 time before attack was not significantly different between batches. Once contacted, the mussel was initially inspected around the gape between valves. In experiment 1, for mussel-experienced dogwhelks, inspection time was significantly shorter for the gape insertion than for the bored hole attack method (Mann-Whitney - Wilcoxon test: $W = 637.0$, $N_1 = 17$, $N_2 = 26$, $P < 0.05$). Gape insertion attacks by mussel-inexperienced dogwhelks were too few for statistical analysis, but again tended to involve shorter inspection time than bored hole attacks (Table 2). When attacking by the bored hole method, inspection time was not significantly different between mussel-experienced and mussel-inexperienced dogwhelks.

Direct observation together with headphone monitoring of radular activity suggested that radular activity began within 1-5 min after inspection (Fig. 1). Consequently, for all batches, there was no significant difference between inspection time and pre-drilling time.

Drilling activity pattern differed between attack methods (Table 3). In several gape insertion attacks no radular activity was recorded, but since low resolution of the

signal could not be excluded as the cause, these cases were not used in computing the values reported in Table 3. The remaining data showed that gape insertion attacks required less drilling activity, measured both as drilling time and as number of radular rasps. For dogwhelks experienced with mussels in experiment 1, drilling time for the gape insertion attack method, 14.06 ± 2.77 h, was significantly shorter than for the bored hole method, 57 ± 3.56 h (Mann-Whitney - Wilcoxon test: $W = 260.0$, $N_1 = N_2 = 13$, $P < 0.0001$). Similarly, the ratio drilling time / handling time was significantly less for the gape insertion method, 0.28 ± 0.04 , than for the bored hole method, 0.56 ± 0.02 ($W = 99$, $N_1 = N_2 = 13$, $P < 0.005$). Number of radular rasps was marginally not significantly less for the gape insertion method than for the bored hole attack method ($W = 211.0$, $N_1 = N_2 = 13$, $P = 0.07$). Consequently, the intensity of radular activity, i.e. the ratio number of radular rasps / drilling time, was significantly less for the bored hole method, 41.74 ± 8.93 radular rasps per hour, than for the gape insertion method, 107.28 ± 16.15 rasps h^{-1} ($W = 123.0$, $N_1 = N_2 = 13$, $P < 0.01$).

Table 3. Drilling parameters, for mussel-experienced and mussel-inexperienced dogwhelks

Attack method	Experiment 1				Experiment 2		
	Experienced		Inexperienced		Experienced		Inexperienced
	hole	gape	Hole	gape	Hole	gape	hole
N	13	13	15	2	12	14	11
DT (h)	57.99 ± 3.56	14.06 ± 2.77	56.12 ± 4.51	12.15 ± 5.04	55.80 ± 4.08	12.62 ± 4.08	54.38 ± 4.29
NRR	2128 ± 334	1753 ± 657	4191 ± 895	870 ± 271	3976 ± 1062	1606 ± 714	3409 ± 884
NRR/DT	41.74 ± 8.93	107.28 ± 16.15	79.76 ± 18.47	97.84 ± 63.01	71.26 ± 16.47	124.80 ± 29.03	60.93 ± 13.56
DT/HT	0.56 ± 0.02	0.28 ± 0.04	0.57 ± 0.02	0.22 ± 0.04	0.62 ± 0.03	0.31 ± 0.09	0.61 ± 0.05
PreDT (h)	3.09 ± 1.62	1.44 ± 0.64	1.65 ± 0.37	3.20 ± 1.80	1.37 ± 0.36	1.72 ± 1.06	1.05 ± 0.24
INGT (h)	42.52 ± 0.27	31.87 ± 3.45	39.97 ± 4.00	37.25 ± 6.34	31.77 ± 2.89	24.85 ± 3.67	38.80 ± 10.55

Values are means ± SE. DT = drilling time; NRR = number of radular rasps; NRR/DT = ratio between NRR and DT; DT/HT = ratio between DT and handling time; preDT = pre-drilling time; INGT = ingestion time; hole = penetration through a drilled hole; gape = penetration between the gaping valves.

Drilling time for the bored hole attack method was not significantly different between mussel-experienced and mussel-inexperienced dogwhelks (experiment 1: $W = 201.0$, $N_1 = 13$, $N_2 = 15$, $P = 0.58$; experiment 2: $W = 143.5$, $N_1 = 12$, $N_2 = 11$, $P = 1$). However, in experiment 1, radular activity was marginally not significantly less for mussel-experienced dogwhelks than for mussel-inexperienced (number of radular rasps: $W = 149.0$, $N_1 = N_2 = 13$, $P = 0.08$; number of radular rasps / drilling time: $W = 148.0$, $N_1 = N_2 = 13$, $P = 0.08$). This trend was not reflected in experiment 2 (number of radular rasps: $W = 153.0$, $N_1 = 12$, $N_2 = 11$, $P = 0.60$).

Mussel shell thickness at the borehole ranged from 0.5 to 0.9 mm and was not significantly different in mussels presented to dogwhelks experienced with mussels and dogwhelks inexperienced (Mann-Whitney - Wilcoxon test: $W = 1617.5$, $N_1 = 45$, $N_2 = 55$, $P = 0.50$). Drilling time was significantly correlated with shell thickness only for mussel-inexperienced dogwhelks of experiment 1 (Pearson correlation: $r = 0.645$, $N = 15$, $P < 0.01$). Penetration rate, estimated as shell thickness / drilling time was 0.29 ± 0.009 mm day⁻¹ for all experimental batches. Handling time was significantly correlated with shell thickness for dogwhelks inexperienced with mussels in experiment 1 (Pearson correlation: $r = 0.582$, $N = 30$, $P < 0.01$) and for mussel-experienced dogwhelks in experiment 2 ($r = 0.677$, $N = 19$, $P < 0.002$).

For dogwhelks experienced with mussel in experiments 1, ingestion time was shorter when using the gape insertion attack method (31.87 ± 3.45 h) than when using the bored hole method (42.52 ± 0.27 h), the difference being marginally non-significant (Mann-Whitney - Wilcoxon test: $W = 214.0$, $N_1 = N_2 = 13$, $P = 0.05$).

2.5. Discussion

The technique used for monitoring drilling activity has allowed, for the first time, the different components of prey handling by dogwhelks to be distinguished. This in turn has enabled the effect of dietary experience on the prey-handling process to be examined in greater detail.

Greatest behavioural contrast was found in experiments 1 and 3, between dogwhelks with different field-based experience of mussels: those from Red Wharf Bay, where mussels and barnacles were abundant, more readily adopted the faster,

gape insertion attack method than those from Menai Bridge, where only barnacles were present. We believe that dietary experience is the principal factor explaining the higher occurrence of the shorter handling method used by dogwhelks from the mussel-dominated shore. Even though risk of predation is higher at Menai Bridge, a sheltered shore with high crab density (personal observation), dogwhelks from that shore did not use the shorter handling method. Although Red Wharf Bay is a moderately exposed shore (with relatively lower crab density), wave impact is unlikely to pose a major risk to foraging dogwhelks. We therefore discount risk of predation or dislodgement by waves as potentially confounding factors in our interpretation of population differences in prey-handling behaviour. Empty mussel valves, whose posterior margins show signs of drilling that characterize the gape insertion attack method, frequently are found among smaller size classes on mussel-dominated shores (personal observation; Hughes & Burrows 1993). Since, in the present experiments, mussels were almost totally consumed, switching from the bored hole attack method to the faster gape insertion method doubled the feeding rate. While potentially doubling the energy gain per foraging bout, the reduction in handling time also could halve the exposure time to environmental hazards, so promoting fitness under many commonly occurring field conditions (Burrows & Hughes 1990).

Although in experiments 1 and 3 the gape insertion attack method was used more frequently by dogwhelks already experienced with mussels in the field, it was never used exclusively. When mussel-experienced dogwhelks used the alternative bored hole attack method they took just as long as mussel-inexperienced individuals to drill the prey. This matches Hughes & Dunkin's (1984) finding that whereas dogwhelks from a barnacle-dominated shore learned to increase efficiency in handling smaller mussels by drilling thinner parts of the shell, they did not do so for larger mussels spanning the size range used in the present experiments. Nevertheless, dogwhelks experienced with mussels performed fewer drilling clusters than inexperienced dogwhelks. This difference becomes significant with a one-tailed test, which is justified if it is considered that experience could only increase foraging efficiency. Drilling time was conserved perhaps through changes in the efficiency of chemical dissolution, used alternately with scraping (Carriker 1981). Whether or not this reduction in radular activity makes significant savings in energy cannot be assessed without appropriate metabolic data.

In contrast to experiments 1 and 3, laboratory-based experience of mussels had no significant effect on the frequency of attack methods used by dogwhelks in experiment 2. Many studies have addressed the effect of experience on prey-handling behaviour (review in Hughes et al. 1992; see also Hughes & Croy 1993; Serra et al. 1997) and in most of them increasing experience with a particular type of prey resulted in greater handling efficiency. Typically 5-10 attacks are required for handling skills to reach an asymptote in animals as diverse as fishes, crabs and snails. Since similar numbers of prey were attacked by dogwhelks in experiment 2, it is puzzling why handling efficiency showed no significant improvement. Possibly, learning to adopt the gape insertion attack method requires a longer sequence of encounters with small-to-medium sized mussels.

The gape insertion method presumably relies upon effective application of toxin (Andrews 1991) to the mantle rim exposed by initial drilling. Most gape insertion attacks in the present study were performed with little drilling and evidence of scraping often was only apparent under the dissection microscope. Drilling therefore may be limited to the first exposure of tissue, when toxin can be applied. Video analysis revealed that the valves of the mussels were gaping slightly during ingestion, presumably as a result of narcotization. It is unlikely that the accessory salivary glands hold a sufficient dose of toxin to be effective on large mussels, a conclusion corroborated by experiment 1 where gape insertion attacks were performed by significantly larger dogwhelks (see also Serra et al. 1997). Encounters with prey unsuitable for the gape insertion method may reinforce continued adoption of the bored hole method, which ultimately is successful with any prey and so is more suitable as a general attack strategy.

A process analogous to the gape insertion method is used by dogwhelks experienced in attacking barnacles, where penetration becomes focussed on sutures between the skeletal plates (Hughes & Dunkin 1984). Behavioural selection of the penetration site, however, probably differs according to the morphological disparity between barnacles and mussels, rendering skills non-transferable (Osgood 1949) between these prey types. Long-term dietary history may have caused a lasting behavioural effect that could not be altered by the relatively short-term experience staged in the laboratory. Behavioural traits also might be genetically controlled (Hughes & Taylor 1997), perhaps hindering adjustment to alien prey. If so, here might

be clear evidence of small scale genetic differentiation in dogwhelk populations as a consequence of direct development and localized differences in chromosome number (Dixon et al. 1994). Laboratory rearing experiments will be required to resolve this problem.

The present study illustrates the value of adopting a mechanistic approach to the analysis of foraging behaviour, where an understanding of decision-making processes requires detailed knowledge of ways in which constraints and state variables influence sub-components of the prey-handling process (Burrows & Hughes 1991; Houston 1993).

Chapter 3. Automatic recording of the radular activity of dogwhelks drilling mussels

3.1. Abstract

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 5 rasps per min). Accordingly, the total amount 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.

3.2. 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 (reviews in Carriker & Smith 1969; 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.

N. 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.

3.3. Methods

Adult dogwhelks, ranging from 30 to 40 mm of 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. 1999a).

Mussels 25~30 mm in shell length were collected every two weeks from a sandy beach near Bangor (North 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 sea water which was replaced with freshly collected sea water every two weeks.

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 10x10 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 one audio-listened as 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. 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 1 frame every 3 sec.

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 1~8 unit events, each cluster lasting 0.7~1 sec and separated by gaps of 1.3~1.6 sec, 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 distribution 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., 1999a for more details). Each drilling sequence was thereby arbitrarily divided in four temporal segments of equal duration (one fourth of DT, see Fig. 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 period, in turn estimated by the ratio DT/periodicity. Thus the parameter is given by the formula (EDT x 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 ANALYSIS OF VARIANCE, using the Huynh-Feldt epsilon to reduce degrees of freedom to accommodate for non-sphericity (Norušis 1993).

3.4. Results

Time intervals between unit events of radular scraping followed a bimodal frequency distribution (Figure 1). Intervals grouped around 0.2~0.3 sec corresponded to lapses between bumps of radular teeth within a single rasp (intra-rasp time), whereas those grouped around 1.3~1.6 sec corresponded to intervals between the last event of a rasp and the first event of the following rasp (inter-rasp time). The lack of intervals between the two modes (0.7~1 sec) represented the temporal criterion for separating intra- and inter-rasp events by the data processing system.

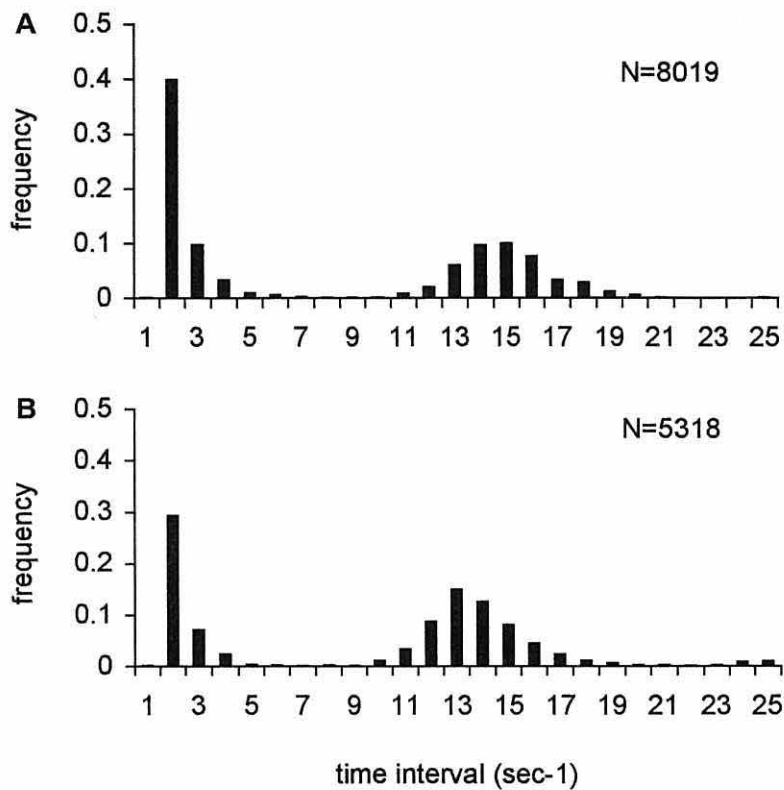


Figure 1. Frequency distribution, for a range of 2.5 sec, of time intervals between successive unit events of radular rasping during two drilling processes by dogwhelks on mussel shells, A and B. Each unit event probably correspond to the application of one row of radular teeth to the mussel shell. Intervals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

Twenty-one attacks were analysed for radular activity and for all of them the correspondance 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.

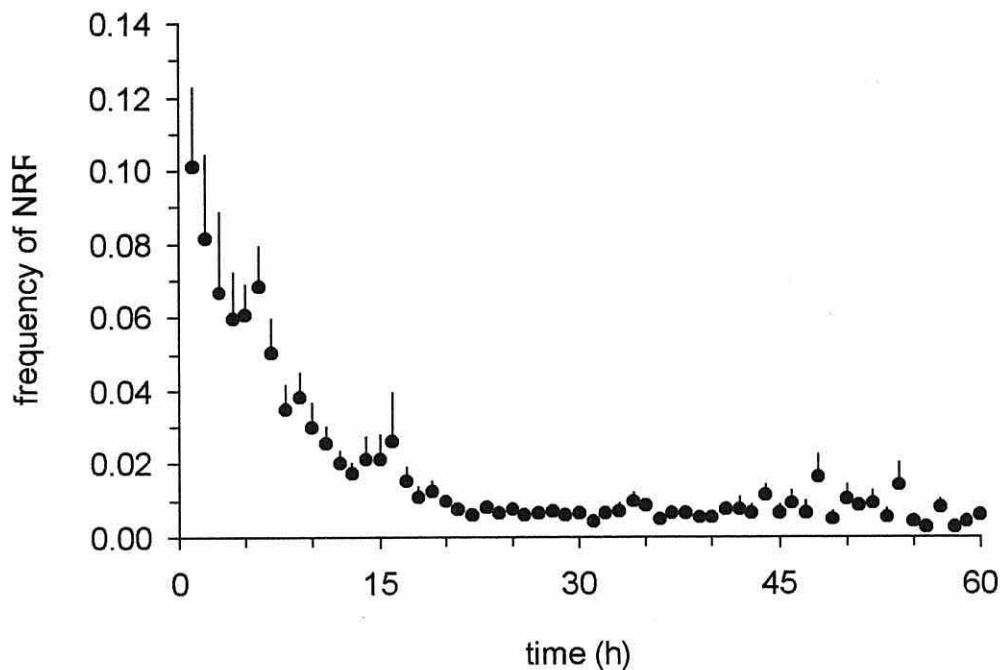


Figure 2. Frequency distribution of Number of Radular Rasps h^{-1} recorded for 21 dogwhelks drilling on mussel's shells. Values are plotted along a 60 h range, during which period the shell penetration process generally is accomplished. Data are means (+SE).

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.* 4 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 1–3 of shell penetration (30, 46 and 84 min, respectively), followed by a slight decrease in phase 4 (71 min).

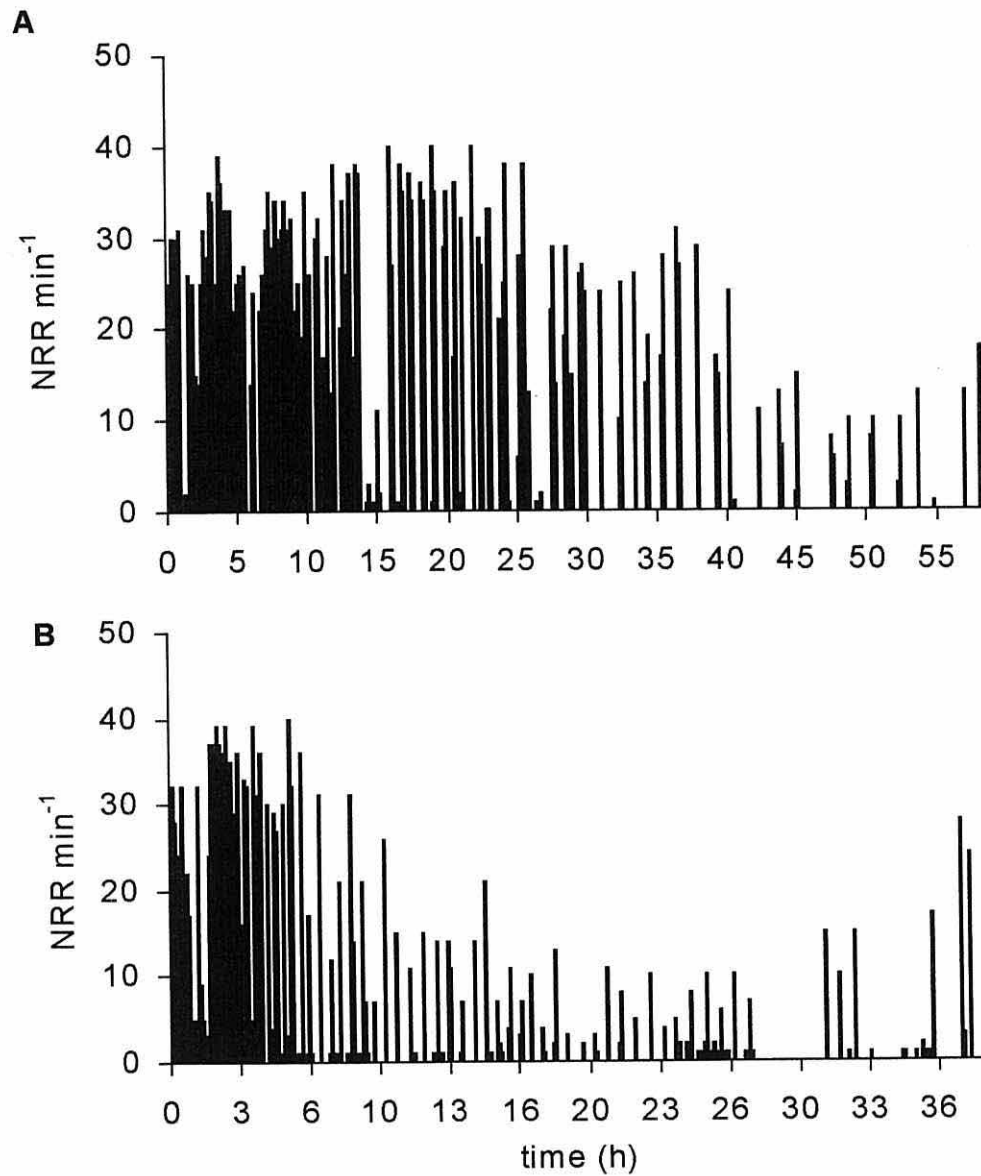


Figure 3. Time series of Number of Radular Rasps min⁻¹, plotted for two drilling sequences, A and B, throughout the drilling process. Each sequence is divided into four segments (see text for explanation) of equal duration, as shown for the attack A.

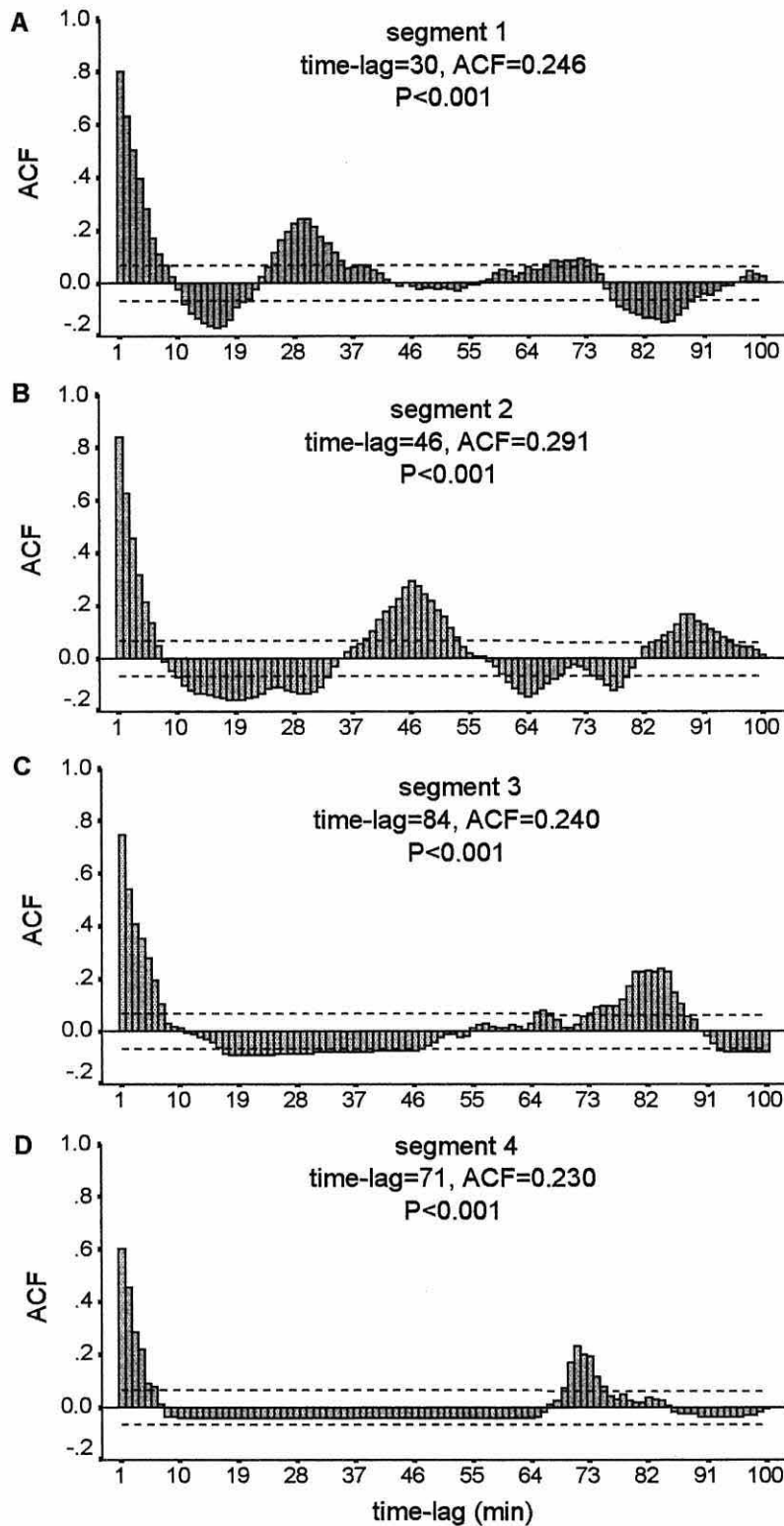


Figure 4. Values of autocorrelation coefficient (ACF) from the analysis of each temporal segment of the drilling sequence shown in Figure 3A. Horizontal dashed lines show 95 % confidence limits. Time lags (min) corresponding to the higher ACF are reported in the charts, and represent the significant 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).

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 2–4 (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 6 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 , $F=2.71$, $P=0.06$).

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.

Parameter	Temporal segment				<i>P</i>
	1	2	3	4	
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 ± standard error; *P* is the probability that means are different across the four temporal segments, tested by repeated measures ANOVA.

3.5. 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 inter-rasp 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 sec and separated by 1~3 sec), 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 the more general investigation of prey-handling behaviour (see Rovero et al. 1999a). 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). The occurrences of radular activity and, indirectly, of chemical application, are in qualitative agreement with drilling profiles reported for U. 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, we 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.

Chapter 4. Cardiac and behavioural responses of mussels to risk of predation by dogwhelks

4.1. Abstract

We used non-invasive, infrared phototransducers to continuously record heart beat of common mussels, *Mytilus edulis*, experiencing successive phases of interaction with the predatory gastropod *Nucella lapillus*, from initial threat to attack and consumption. Coupling physiological monitoring with behavioural analysis allowed responses of mussels to predation threat to be investigated in detail. Compared to values of normal feeding activity, heart rate significantly increased when mussels were in the presence of effluent from dogwhelks. When attacked by dogwhelks, heart rate of mussels increased further, together with enhanced rate of gaping of valves. Considering the heart rate as a reliable estimator of respiratory function, these cardiac responses might be a mechanism to compensate increased energy demand in order to cope with predation hazard. If so, the theoretical importance of trading-off energy balance against risk of predation is supported by our results. Cardiac and behavioural responses varied throughout the attack according to the penetration method adopted by dogwhelks. When mussels were attacked through a drilled hole, heart rate tended to increase and periods of cardiac pausing appeared close to the point of death. On the contrary, mussels attacked by penetration between the valves showed decreasing heart rate throughout the attack, together with relatively earlier cessation of valve gaping and appearance of cardiac pausing. These differences clearly support the hypothesis that dogwhelks penetrating between the valves are able to induce muscular paralysis of prey by injection of toxins.

4.2. Introduction

Monitoring heart rate has been used extensively to study physiological responses of invertebrates to environmental fluctuations. For bivalve molluscs and other marine taxa in particular, investigations have addressed the effects of physico-chemical variables, such as temperature, salinity, exposure to hypoxia and air (see Bayne et al.

1976; DeFur & Mangum 1978; Shick et al. 1986; Marshall & McQuaid 1993). Few data are available, however, on physiological responses of subjects to perceived risk of predation (but see Metcalf et al. 1987) or even to actual predatory attack. On the other hand, predation threat has been the object of much behavioural research, which theoretically and experimentally has shown how predation risk constrains the decision-making processes of prey (reviews in Lima & Dill 1990; Godin 1990; Sih 1993). Attempts to estimate the fitness consequences of coping with predation threat have been made by quantifying changes in the time-budget or behavioural efficiency, for example involving refuging, increased vigilance and associated reductions in foraging opportunity (e.g. Milinsky & Heller 1978; Abrahms & Dill 1989; Vadas et al. 1994; Serra et al. 1997). An ideal experimental approach, however, would require behavioural responses to be monitored together with one or more estimators of the prey's physiological status, such as heart rate. This would allow the physiological demands of different responses to be directly inferred and therefore fitness consequences analysed in greater detail. New techniques for non-invasive monitoring of physiological states of selected invertebrates (see Depledge & Andersen 1990; Aagard et al. 1991) potentially allow the above approach to be achieved, since animals can be monitored while performing normal activity. In the present study, this has been attempted by monitoring in the laboratory both cardiac and behavioural responses of common mussels (*Mytilus edulis*) under successive phases of the interaction with the predatory muricid gastropod *Nucella lapillus*, from initial perceived risk of predation to final attack.

The common mussel is widely distributed in the high and mid-intertidal of northern hemisphere shores (Seed 1976). The cardiac physiology of *M. edulis* has been studied for many years using invasive techniques such as electrode implantation or visual inspection through a hole drilled through the shell (review in Bayne et al. 1976; see also Shick et al. 1986). Only recently has the application of a non-invasive technique been tested on this species (Haefner et al. 1996). Although the relationships between heart beat and metabolic rates are still not fully understood (Booth & Mangum 1978; Famme 1981), it is generally accepted that for mussels heart rate is a reliable estimator of respiratory function (Bayne 1971; Coleman 1974; Marshall & McQuaid 1993).

The dogwhelk, *N. lapillus*, is among the major predators of mussels (Seed 1976)

and readily attacks prey in the laboratory (Hughes & Dunkin 1984). The prey-handling process can last several days, since it involves drilling the prey's shell before ingesting, although the process can be greatly shortened by inserting the proboscis through the gaping valves (Rovero et al. 1999a).

4.3. Methods

General methods

Mussels, 28-32 mm in shell length, were collected from a sandy beach near Bangor (North Wales) and maintained for a month, prior the beginning of the experiments, in aquaria on a diet of *Rhinomonas reticulata*. This prolonged maintenance in controlled conditions was planned to avoid any possible endogenous variation of heart rate during experiments, due to tidal and/or diurnal rhythms (Rovero, unpublished data). The size chosen was large enough for the application of transducers (see below) and is commonly accepted by adult dogwhelks both in the laboratory (Hughes & Dunkin 1984) and in the field (Hughes & Drewett 1985).

Adult dogwhelks, 35-40 mm in shell height, were collected from Red Wharf Bay, a rocky shore on Anglesey (North Wales) densely populated by mussels. Each dogwhelk was deprived of food for 2 weeks to standardise hunger level, and then monitored while attacking a mussel. In the field, dogwhelks may endure even longer periods without feeding, particularly during harsh environmental conditions (Burrows & Hughes 1991).

Experiments were conducted from September to December 1998 at 17 ± 1 °C under continual illumination. Experimental aquaria (25 x 40 x 30 cm³) contained 10 l of recirculating, aerated sea water regularly supplied with *R. reticulata* to give an almost constant food concentration of about 500 cells/ml.

Techniques

The non-invasive technique for monitoring heart rate was similar to that introduced by Depledge & Andersen (1990). The sensor consisted of a phototransistor axially coupled with an infrared light-emitting diode, that was fixed with cyanoacrylate glue to the shell of the mussel in the dorsal and marginal position corresponding to the heart (Fig. 1). After filtration and amplification, the signal was transmitted to a

computer (see Santini et al. 1999) in two alternative ways: (a) by a digital oscilloscope (Virtual Bench software) which allowed instantaneous and graphic visualisation of heart beats, or (b) by a Quick Basic program, which captured voltages every 0.1 s, throughout set periods of time, allowing long-term storage of data. The first method was used prior to each recording session to check the correct position of the sensor on the shell and the second method was used for recording data during the experiments. For each interval of continuous recording, a sequential file was automatically stored and exported for processing. Since we monitored the heart beat of mussels up to the point of death, we expected to record arrhythmic or temporary arrested heart rates. We therefore processed data by statistical determination of rhythmicity of the heart rate. Autocorrelation analysis for time-series data (Diggle 1990) was applied to each period of continuous recording in order to test whether or not heart rate was significantly periodical ($P < 0.001$). The periodicity, if any, was estimated by the particular time-lag corresponding to highest value of the autocorrelation coefficient. This procedure, therefore, allowed us to detect those recordings, near to the death point of mussels, for which heart beat was either arrhythmic or absent. If periodicity was detected, time-lag values of heart beat frequency were converted to beats min^{-1} .

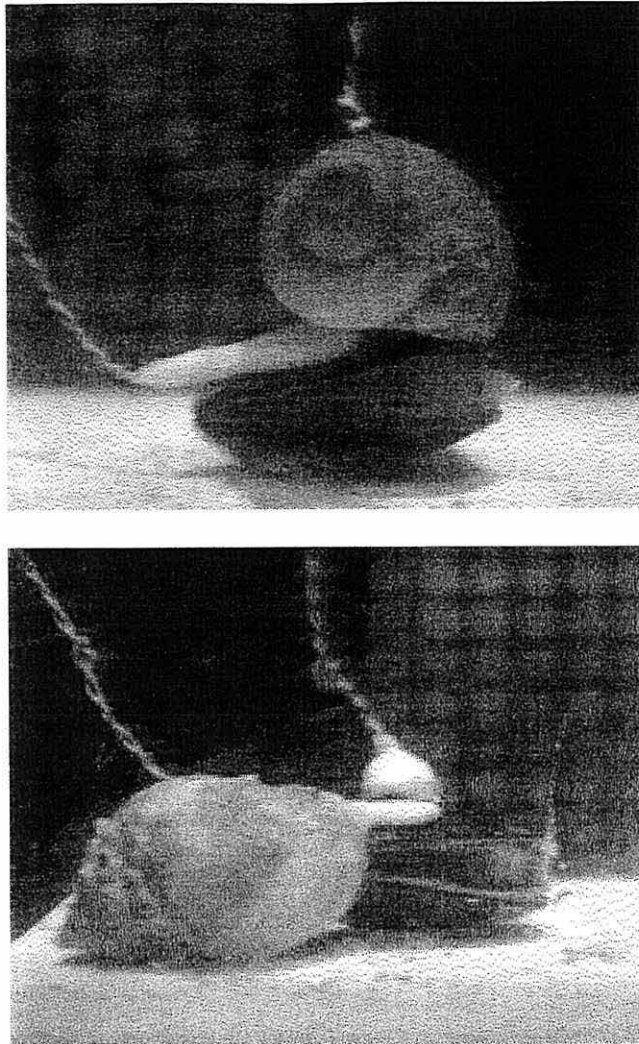


Figure 1. Photographs of mussels under two different attack methods by dogwhelks: by drilling a hole through the shell of the mussel (upper photograph) and by penetration between the gaping valves (lower photograph). Transducers are glued to the superior valve of the mussel. The elongated one (upper photograph) is a piezoelectric transducer for recording radular activity by dogwhelk, the cubic one (lower photograph), closed to the dorsal hinge, is a phototransducer for monitoring heart beat.

Methodological details for recording the radular activity of dogwhelks attacking mussels are reported in Parpagnoli & Chelazzi (1995) and Rovero et al. (1999a). Piezoelectric transducers were fixed to the same valve bearing the heart rate sensor, close to the dorsal hinge (Fig. 1). The other valve was glued to a PVC base, facilitating video-monitoring of behavioural patterns. This isolation and unnatural

position of the prey neither alters the attack methods used by dogwhelks (see below) nor the responsiveness of the mussels (Rovero et al. 1999a). Data were stored on a computer and exported after each attack for processing. We obtained profiles of drilling activity, throughout the shell penetration process, quantified as the number of radular rasps per h, each rasp corresponding to a series of strokes of the radular teeth on the substratum.

Heart rate and drilling recordings were coupled with video-monitoring of the animals by a camera placed beside the aquarium, connected to a time-lapse video-recorder that stored one frame every 3 s.

Experimental Procedures and Analysis of Data

Experiment 1: Effect of Predation Threat on Heart Rate

To monitor cardiac rates during the normal feeding activity, 8 mussels equipped with heart rate sensors were placed in the aquarium and heart beats sequentially recorded during 1h, divided in 60 sec of continuous recording every 5 min. Mussels were first allowed to acclimatise to experimental conditions for about 30 min, until they assumed the gaping posture (i.e. open valves) indicating active ventilation (Bayne 1971) and heart function (Coleman 1974). After these trials, 50 dogwhelks were placed in the same aquarium inside a 10 x 10 cm compartment made of plastic mesh and left for 12 h together with the mussels. Mussels were then monitored following the same procedure as described above. The experiment lasted 2 days.

Mean heart rate of each mussel was computed for both treatments; values were analysed together with those for the same mussels under the first 3 h of predatory attack (see experiment 2). Means were compared by repeated measures analysis of variance (Sokal & Rohlf 1995).

Experiment 2: Heart Rate Under Predatory Attack

A mussel bearing both sensors was placed together with a dogwhelk into a 10 x 10 cm compartment within the aquarium. Each predatory sequence was monitored from the beginning of inspection to abandoning of the consumed mussel once the dogwhelk had completed the attack (lasting about 90 h for bored hole attacks and 45 h for gape insertion attacks, see below for a description of attack methods). The predator/prey pair was then replaced with another. These experiments were run over a period of 10

weeks.

Data from video, drilling activity, and heart rate monitoring were analysed and integrated as follows. From video monitoring, attacks were classified by method, according to whether the mussel was penetrated by drilling a hole through the shell (bored hole attack method) or by insertion of the proboscis between the gaping valves (gape insertion attack method) (see Rovero et al. 1999a and Fig. 1). Behaviour of mussels was analysed for gaping rate (i.e. number of gapes per h, each gape being a closure of the valves followed by immediate re-opening). Since for high gaping rates the exact values were not discernible, gaping rate was expressed on an ordinal scale: 0 = non-gaping; 1 = 1-9; 2 = 10-19; 3 = 20-49; 4 = 50-99; 5 = >100 gapes h^{-1} . When no, or little, gaping activity occurred, it was noted whether the valves were kept open or closed.

Prey handling by dogwhelk consists sequentially of inspection, drilling and ingestion phases (Hughes & Dunkin 1984). Monitoring of drilling activity allowed these phases to be distinguished (Rovero et al. 1999a) and so heart rate of mussels could be categorised for each phase separately. The drilling phase was defined as the period characterised by radular activity and subsequent heart rate values were considered to represent the ingestion phase.

Heart beats of mussels were recorded for 30 s every 15 min throughout the predatory attack and mean hourly values of heart rate were computed. Those periods for which no significant rhythmicity was detected by autocorrelation analysis were considered to represent temporary cardiac pausing (heart rate = 0) and scored separately as % pausing h^{-1} . The death of mussels was judged by the continuous recording of cardiac pausing. Individual profiles combining heart rate and % pausing were plotted together with behavioural profiles showing gaping rate and mutual position of valves (open or closed).

Profiles of heart rate also were pooled for all attacks, grouped by attack method. Regression analysis was used to visualise trends. Slopes were compared by repeated measures analysis of variance. To further analyse differences between attack methods, variations in the length of attacks were normalised by dividing each attack into 4 equal temporal segments, for which mean values of cardiac and behavioural parameters were computed (heart rate, % pausing h^{-1} , index of gaping rate and fraction of hours with closed valves). Differences of these parameters among temporal

segments and between attack methods were tested by repeated measures analysis of variance, using the Huynh-Feldt epsilon to reduce degrees of freedom to accommodate for non-sphericity (Norušis 1993).

Experiment 3: Temporal Dynamics

The design of experiments 1 and 2 was constrained by the available recording apparatus. Thus, heart rate could be continuously recorded for only one mussel at a time and even instantaneous readings, switching between subjects, could be taken for no more than 8 mussels within an acceptable time period (5 min). Therefore, pre-treatment and treatment phases had to be run sequentially, as in experiment 1. Experiment 3 was designed to test the independence of changes in heart rate and residence time of mussels in aquaria, by using a pre-treatment period equalling the total duration of both experiment 1 and 2. The experiment also examined in greater detail the temporal dynamics of heart rate resulting from temporary exposure to dogwhelk effluent.

We placed 8 mussels in an aquarium as in experiment 1, but in the presence of an empty cage. We monitored heart rate of each mussel over 3 days, making instantaneous readings twice per day. We then placed 50 dogwhelks in the cage and made instantaneous recordings of heart rate once per hour for the first 12 h, and once again at 24 h. We then removed dogwhelks from the cage and changed the water. To maximise removal of effluent, untreated water was allowed to circulate in the aquarium for about 10 min. Heart rate was then monitored twice per day for 2 more days, as in the pre-treatment phase. The experiment lasted 1 week.

A profile of mean heart rate per day was plotted for the pre-treatment, treatment and post-treatment phases, together with a more detailed profile of heart rate during exposure to effluent. Values were compared by repeated measures analysis of variance and by Wilcoxon's signed-ranks test (Sokal & Rohlf 1995).

4.4. Results

Mean heart rate increased from 23.10 ± 0.61 beats min^{-1} (mean \pm SE) during normal feeding activity to 29.82 ± 0.91 beats min^{-1} in similar conditions but in the presence of effluent from dogwhelks (Fig. 2a). Interestingly, this variation was not followed by

any variation in the gaping rate, since mussels kept the valves almost constantly open in both treatments. When in contact with the predator heart rate of the same mussels increased further, to 36.84 ± 1.59 beats min^{-1} , concomitant with an increase in the gaping rate (see below). Differences in heart rate among the three treatments were significant (repeated measures ANOVA: $F_{2,14} = 65.90$, $P < 0.001$). Examples of heart beat profiles, for one mussel under different treatments, are shown in Fig. 3. The increase in heart rate from treatment 1 to treatment 3 is accompanied by an increase in the voltage amplitude of the signal.

In experiment 3, heart rate of mussels did not vary significantly during pre-treatment phase (Fig. 2b; repeated measures ANOVA for 1-3 days: $F_{2,14} = 2.38$, $P > 0.05$). After 1 h of exposure to dogwhelk effluent, heart rate increased significantly (Fig. 2c; Wilcoxon test between the last h of pre-treatment and the first h of treatment: $N_1 = N_2 = 8$, $Z = -2.37$, $P < 0.02$), showing a peak after 2 h and then stabilizing about a mean of 31.15 ± 0.09 beats min^{-1} , without any significant variation until at least 24 h from first exposure to effluent (repeated measures ANOVA for 3-24 h: $F_{8,56} = 1.62$, $P > 0.05$). As noted in experiment 1, mussels did not show any behavioural difference between pre-treatment and treatment phases. When effluent was removed, heart rate decreased slowly, returning after 24 h to values not significantly different from those during pre-treatment (Fig. 2b; repeated measures ANOVA for 1-3 and 6 days: $F_{3,21} = 2.85$, $P > 0.05$).

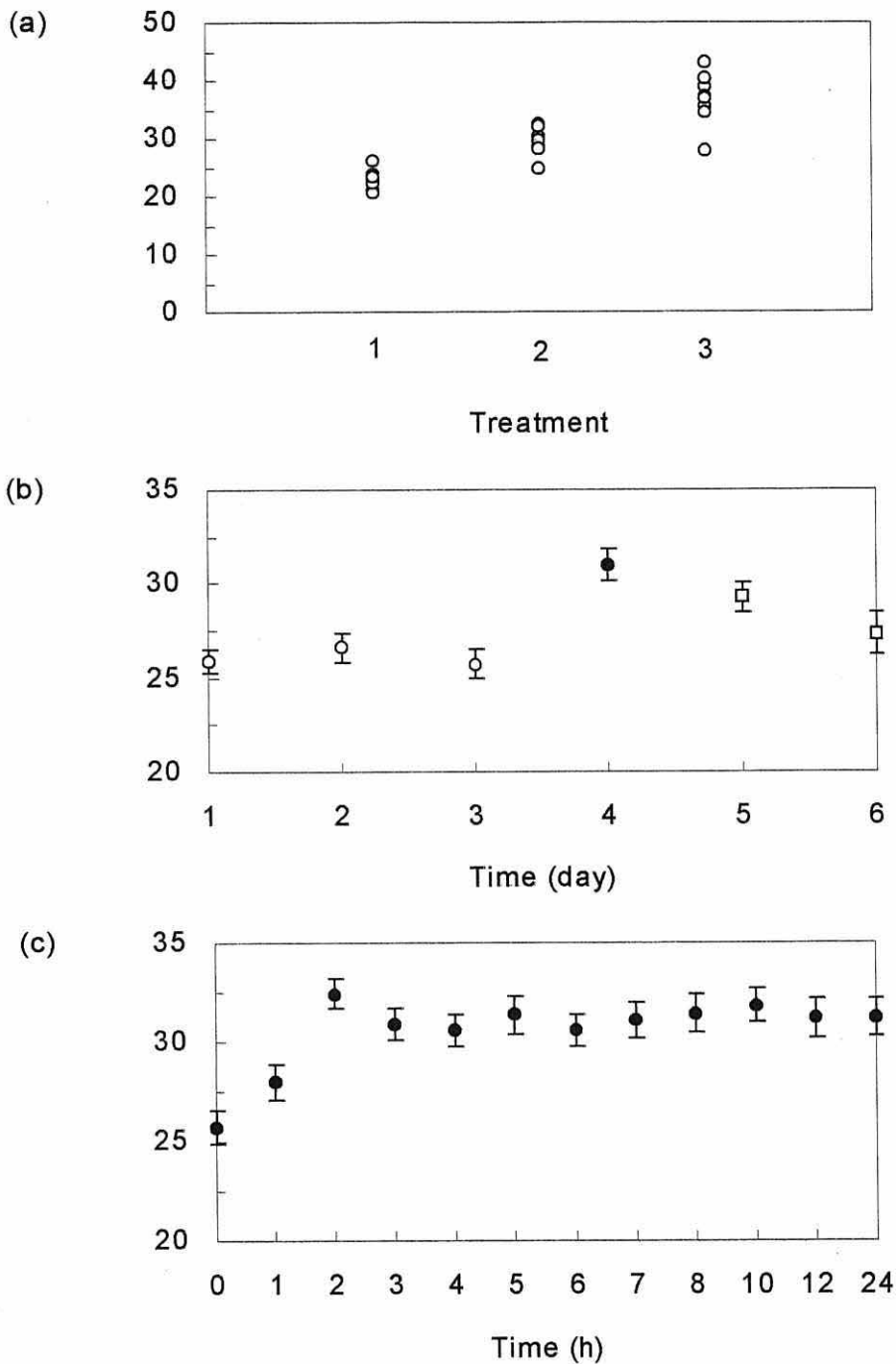


Figure 2. (a) Mean heart rate (beats min⁻¹) of 8 mussels consecutively monitored under three experimental conditions, plotted along the abscissa: (1) during 1 h of normal feeding activity, (2) during similar conditions but with effluent from dogwhelks and (3) during the first 3 h under predation attack by dogwhelks. (b) Mean (\pm SE) heart rate pooled for 8 mussels monitored for 3 days during normal feeding activity (pre-treatment phase, open circles), during 1 day with dogwhelk effluent (treatment phase, closed circle), and during 2 days after removal of dogwhelk effluent (post-treatment phase, open squares). (c) Mean (\pm SE) heart rate of the same mussels as in chart (b) during successive h since exposure to dogwhelk effluent.

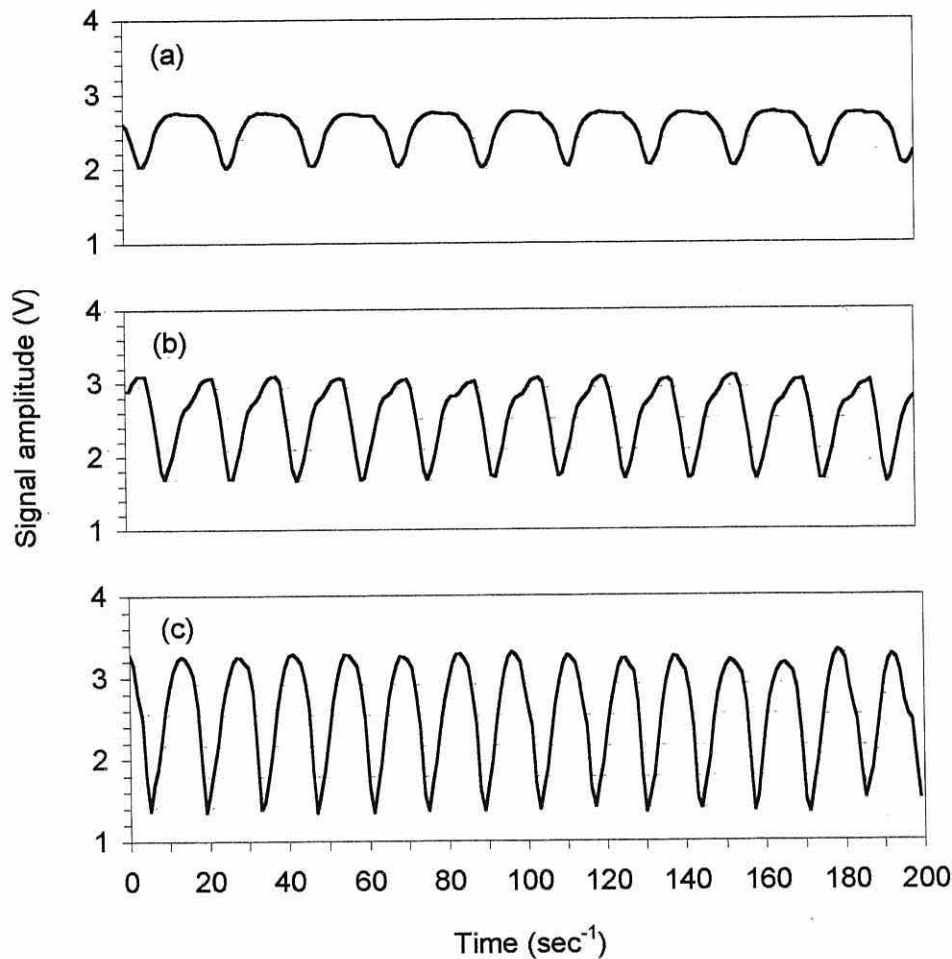


Figure 3. Heart beat profiles, plotted for a period of 20 s, for a mussel monitored under the same three experimental conditions (a, b, c) as explained in the legend of Figure 2.

Responses of mussels to 9 gape insertion attacks and 7 bored hole attacks by different dogwhelks were monitored. Further replication was prevented by the long duration of staging and observing each attack (in the order of 4 days per attack). Profiles of cardiac and behavioural parameters for each method are plotted in Fig. 4 for two individuals. For mussels attacked by the bored hole method, mean heart rate tended to increase throughout the attack and then showed a marked increase after the beginning of ingestion (Fig. 4a). Heart rate was significantly rhythmic during most of the attack, only becoming arrhythmic or ceasing during the final 10 h. Gaping rate was high at the beginning of drilling (20-50 gapes h^{-1}), then stabilised at values of 10-20

gapes h^{-1} , and suddenly dropped to zero about 10 h after the beginning of ingestion. This last period was always characterised by closure of the valves.

When dogwhelks used the gape insertion method of attack, the heart rate of mussels showed a more variable pattern among individuals, and in a few cases decreased throughout the attack (Fig. 4b). Temporary cardiac arrest (pausing) generally appeared relatively earlier than in bored hole attacks. Radular activity did not occur during most attacks by gape insertion and so handling phases could not be distinguished. Gaping rate was high during the first 5 h (50-100 gapes h^{-1}) and cessation of gaping appeared relatively early, concomitant with passively open valves and retraction of the mantle rim.

Hourly values of heart rate were pooled over a range of 22-50 h after the beginning of attack, for gape insertion and bored hole attacks respectively (Fig. 5), since within those periods the mussels were generally killed. Regression analysis confirmed that under the gape insertion attack method heart rate decreased slightly during the predation sequence (least squares linear regression: mean heart rate = $33.50 - 0.12 \text{ h}$, $F_{1,20} = 10.18$, $R^2 = 0.33$, $P < 0.005$), whereas under the bored hole attack method heart rate increased (mean heart rate = $30.79 + 0.07 \text{ h}$, $F_{1,48} = 15.15$, $R^2 = 0.23$, $P < 0.0005$), the rates of change being significantly different between attack methods (repeated measures ANOVA, attack method \times time interaction: $F_{1,49} = 1.65$, $P < 0.05$).

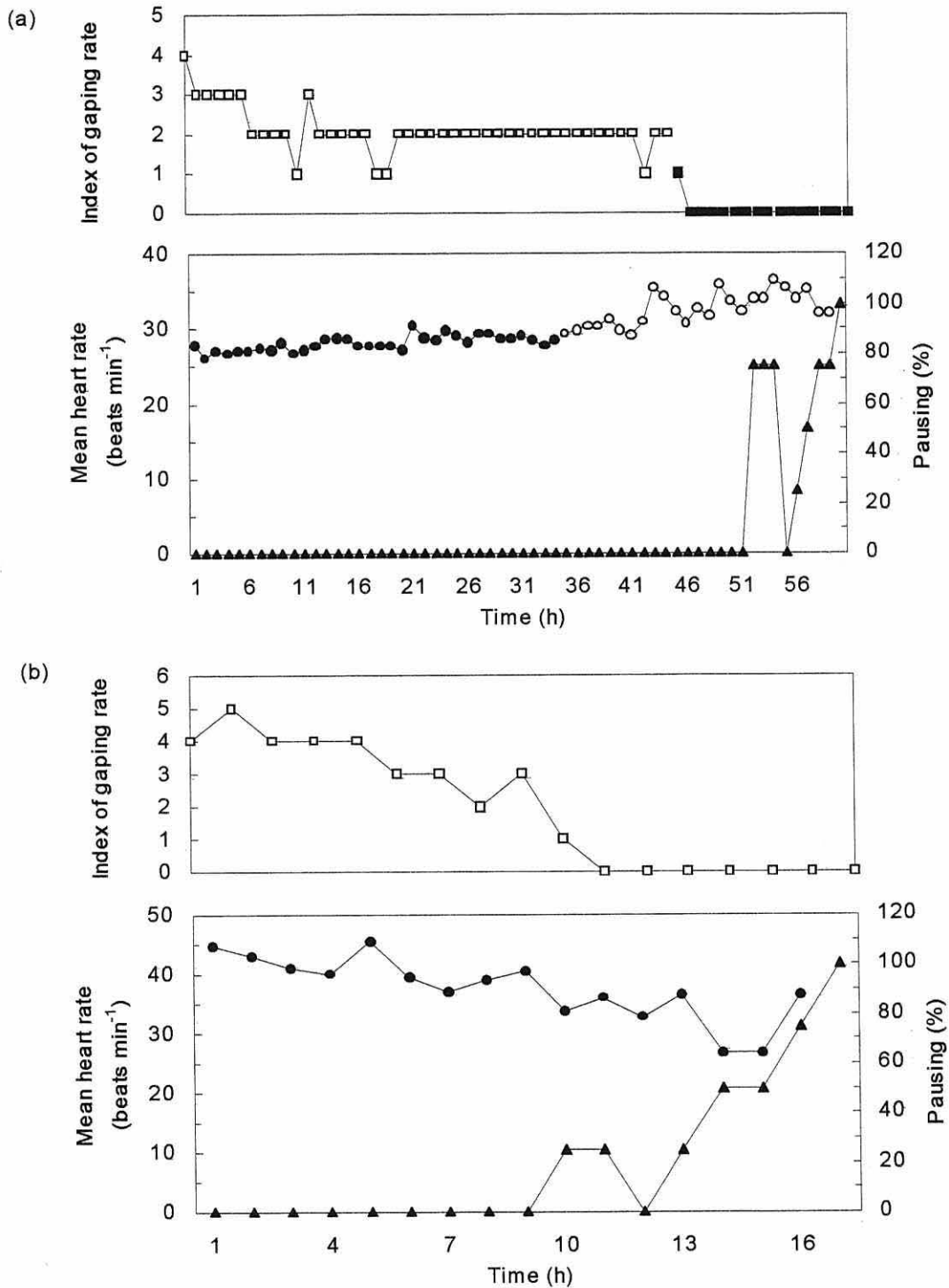


Figure 4. Hourly values of cardiac and behavioural parameters for 2 mussels experiencing different attack methods by dogwhelks: penetration through a drilled hole (a) and penetration by insertion of the proboscis between the gaping valves (b). Values are plotted from the beginning of attack to the point of death of the mussel. For each attack, the first chart shows mean values of an index of gaping activity of the mussel, from 0 (non-gaping) to 5 (> 100 gapes h^{-1}). Open squares mean that the mussel kept the valves open, filled squares mean closure of valves. In the second chart of each attack sequence, heart rate values are categorised according to prey handling phases by dogwhelks, drilling being the period in which radular activity occurred (filled circles) and ingestion the following period (open circles). Note that for the gape insertion attack, radular activity was not recorded and therefore handling phases could not be distinguished. When the cardiac frequency was either arrhythmic or absent (heart rate = 0), data were scored separately and are shown, in the same chart, as % values with cardiac pausing (filled triangles).

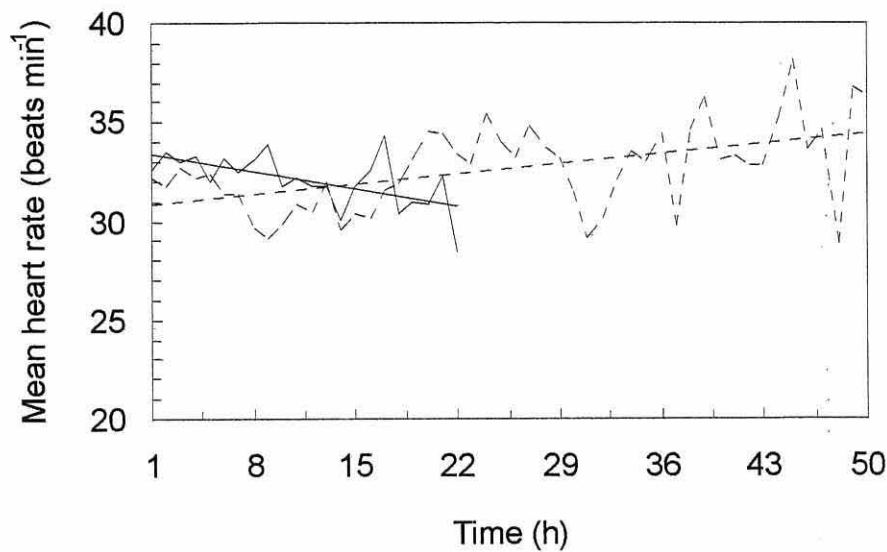


Figure 5. Mean hourly values and regression line of heart rate of mussels experiencing different attack methods by dogwhelks: penetration through the gaping valves (solid line) and penetration through a drilled hole (dashed line). Values are plotted from the beginning of attack for a period of 22-50 h, for gape insertion and bored hole method, respectively, since within that period the mussels were generally killed by dogwhelks.

Heart rate normalised along the temporal scale did not significantly vary either among temporal segments (Fig. 6a, repeated measures ANOVA: $F_{2,4,33.2} = 0.33$, $P > 0.05$) or between attack methods ($F_{1,14} = 0.01$, $P > 0.05$). In contrast, cardiac pausing increased over time, with higher values occurring during the last quarter, before mussels were killed (Fig. 6b, $F_{2,2,30.6} = 14.5$, $P < 0.001$). Cardiac pausing in mussels attacked by the gape insertion method was more frequent than in those attacked by the bored hole method ($F_{1,14} = 9.36$, $P < 0.01$). Gaping rate decreased during the attack (Fig. 6c, $F_{3,42} = 49.14$, $P < 0.001$), differences between attack method being non-significant ($F_{1,14} = 0.12$, $P = \text{ns}$). The proportion of time when mussels had closed valves varied significantly among temporal segments during the attack (Fig. 6d, $F_{1,6,21.8} = 11.44$, $P < 0.01$) and was significantly different between attack methods during the final segment ($F_{1,14} = 29.58$, $P < 0.001$).

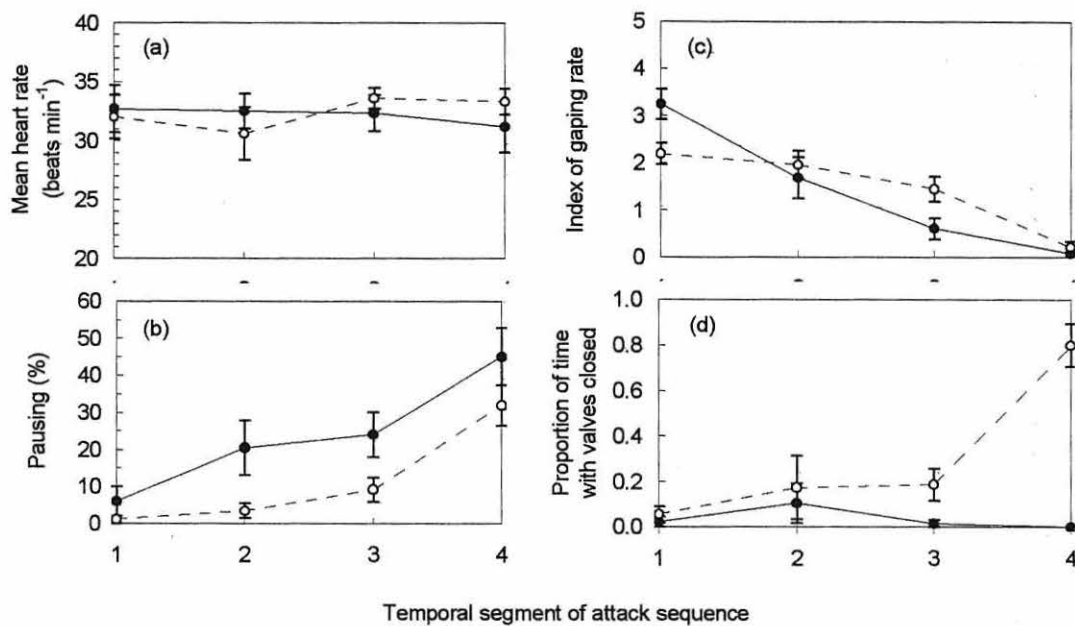


Figure 6. Mean (\pm SE) values of cardiac (a, b) and behavioural (c, d) responses of mussels to predation by dogwhelks. Variations in the length of attacks were normalised by dividing each attack into 4 equal temporal segments. Values are categorised for attack method by dogwhelks, i.e. penetration through a bored hole (open circles, dashed lines) or penetration between the gaping valves (filled circles, solid lines) or. See legend of Fig. 4 for a description of parameters.

4.5. Discussion

The non-invasive technique for monitoring heart rate has enabled, for the first time, an investigation of how risk of predation by dogwhelks is perceived by mussels and of how they respond when under attack.

We believe that the increase in heart rate of mussels after dogwhelks were put into the aquarium, in experiments 1 and 3, was due to an olfactory-mediated perception of predation threat (reviewed in Kats & Dill 1998). Residence time in the aquarium did not influence heart rate of mussels, at least not over a period equivalent to the experimental one. We therefore discount time elapsing from pre-treatment to treatment as a potentially confounding factor in our interpretation of the results. This conclusion also is clearly supported by the relatively sudden increase in heart rate after mussels were exposed to effluent from dogwhelks in experiment 3.

Interestingly, mussels did not show signs of habituation while dogwhelks were

present in the aquarium. Moreover, heart rate remained elevated for a protracted period even after the olfactory cue had been removed. Prolonged refuging or vigilance in response to olfactory signals of predation risk has been noted in several taxa (Kats & Dill 1998). A continued response may be adaptive if return of the predator is likely within a relatively short period after attenuation of the olfactory cue.

Chemical cues from predators are known to induce behavioural responses of prey, such as reduced foraging activity in muricid gastropods (Palmer 1990; Vadas et al. 1994; Serra et al. 1997). In mussels, cardiac response to perceived threat of predation did not appear to be associated with overt behavioural variation, particularly of gaping rates, but represented an alerted physiological state characterised by enhanced heart rate. Without appropriate data it remains unknown whether filtration and respiratory rates varied concomitantly. However, Bayne (1971) suggested that the low oxygen-carrying capacity of the blood of *M. edulis* is compensated by a large blood volume. This would provide an oxygen store that is large in proportion to the mass of respiring tissue. If so, enhancing circulation by increasing heart rate could reflect increased metabolic rate, which might allow the mussels to respond more effectively to a potential attack.

There is evidence that mussels can temporarily immobilize dogwhelks by using byssal threads (Petratis 1987; Day et al. 1991), although it has been demonstrated that dogwhelks can break free from byssal entrapment (Davenport et al. 1996). This potential response of mussels to dogwhelk attack was not observed in our experiments, probably because of the fixed position of mussels. Nevertheless, the increased gaping rate shown by mussels under attack is likely to have a defensive function. Wayne (1980; 1987) describes mussels pinching the foot of whelks by adduction of the shell valves and suggests that repetitive valve closure could have a defensive function, since it forces snails away from the valve edges. This accords well with our results; mussels are initially inspected by dogwhelks around the gape between valves and high gaping rates seem to induce dogwhelks to desist from adopting the gape insertion method, usually shifting to the more time-consuming and risky method of drilling a hole (Rovero et al. 1999a). Consequently, the further increase of heart rate of mussels, between perceiving the risk of predation to facing actual physical attack, again supports the hypothesis that it could represent an adaptive mechanism to compensate increasing energy requirements. In line with

results from strictly behavioural studies (Lima & Dill 1990; Godin 1990), this confirms the theoretical importance of trading-off energy balance against risk of predation (Sih 1993).

At the beginning of the attack, cardiac responses of mussels were very similar irrespective of the penetration method used by dogwhelks. Nevertheless, when the entire predation sequence was examined, the responsiveness of mussels was clearly affected by attack method. Behavioural observations suggested that muscular paralysis occurred when mussels were attacked by dogwhelks using the gape insertion method. This result supports the hypothesis that *N. lapillus* is able to paralyse the prey by injection of toxins secreted by the accessory salivary glands (West et al. 1996). Interestingly, paralysis also involved the heart muscle, both lowering heart rate and causing cardiac pausing, this again matching pharmacological findings (Andrews et al. 1991; West et al. 1996).

Once the attack was initiated, mussels were always eventually killed, therefore it is difficult to assess whether the observed reactions could have a defensive function. The trend of increasing heart rate shown by mussels under drilling attack was concomitant with closure of the valves once ingestion by dogwhelks had begun. This response limits gaseous exchange, causing a limited oxygen supply to tissues (Marshall & McQuaid 1993) and so perhaps accounting for the progressively increasing cardiac frequency. Studies aimed at directly correlate cardiac responses with respiratory variables would be required in order to verify the consistence of these conclusions.

This study shows that non-invasive monitoring of heart rate, while being a reliable technique for physiological investigations (Depledge & Andersen 1990), can also enhance behavioural analysis, particularly when physiological states of animals need to be considered to accurately assess effects of constraining factors.

Chapter 5. Estimating the energetic cost of fighting in shore crabs by noninvasive monitoring of heartbeat rate

5.1. Abstract

Using a noninvasive infrared phototransducer, we continuously monitored the heartbeat rate of resident shore crabs, Carcinus maenas, established individually in separate aquaria, before, during and after fights with intruder crabs. We confirmed that heartbeat rate is a reliable indicator of oxygen consumption and then used it to indirectly estimate the energetic cost of fights differing in duration and intensity. These factors were manipulated through the effects of prior residence and relative size of opponent, predicted from published results with other species. Prior residence in aquaria significantly increased the probability that crabs would initiate fights against intruders. Most fights were resolved by aggressive contacts, display being used extensively only against smaller intruders. Fights between evenly sized opponents and between residents and larger intruders involved almost continuous aggression, whereas fights with smaller intruders involved several shorter bouts of aggression. Fight duration was weakly correlated with the relative size of opponents. Heartbeat rate, measured only in residents, was elevated above resting levels throughout fights, hence energy expenditure during fighting increased linearly with fight duration. Contrary to expectation, heartbeat rate was not significantly influenced by relative size of the opponent or by the intensity of aggression. After fighting, heartbeat rate usually returned to resting levels within 30-60 min, recovery taking longer in fights against larger intruders, when the fight was always lost. We propose that prolonged elevation of heartbeat rate in residents who had lost to larger intruders represented a state of alertness, adaptive against impending risks of resource loss or injury.

5.2. Introduction

Agonistic behaviour is critically related to fitness because for most animals, while potentially endangering survival, it ultimately determines access to limited resources, particularly food and mates (Parker 1974; Archer 1988). It is generally assumed that natural selection optimizes the balance between potential benefits and costs

experienced by contenders, so shaping the evolution of fighting strategies (Maynard Smith & Price 1973; Maynard Smith 1982). In order to develop this cost-benefit approach, it is essential to measure the fitness consequences of alternative behavioural acts (Riechert 1988). One of the most immediate consequences is metabolic cost and attempts to measure this in terms of aerobic and anaerobic metabolism during and after fights, have been fruitful. The methods employed, however, have either been destructive (Thorpe et al. 1995), invasive and therefore potentially stressful (Smith & Taylor 1993), or unable to separately record opponents, whose metabolic rate therefore had to be assumed equal (Hack 1997). A noninvasive technique that allows the metabolic state of individuals to be monitored continually should avoid most of the above problems, so widening scope and enhancing resolution for estimating costs of fighting.

Infrared monitoring of cardiac activity (Depledge & Andersen 1990) is a noninvasive technique widely applied to physiological problems (e.g. Aagaard et al. 1995; Aagaard 1996) and recently extended to behavioural experiments (Rovero et al. 1999b). In the present study, we used this technique to continuously monitor the heartbeat rate of shore crabs, *Carcinus maenas* L., during fights and subsequent recovery. Having established a correlation between oxygen consumption and heartbeat rate, we used the resultant data to estimate the metabolic costs of alternative fighting strategies. To induce different fighting strategies we exploited the behavioural influences of prior residence (Enquist & Leimar 1987), which has not previously been studied in shore crabs but is well-known in a wide variety of territorial animals (e.g. Figler & Einhorn 1983; Englund & Otto 1991), and relative body size (e.g. Maynard Smith & Parker 1976). Crabs are excellent model animals for this purpose, as they fight readily in the laboratory, show various intensities of aggression (Huntingford et al. 1995) and are sufficiently large and suitably shaped for successfully mounting external transducers (Depledge & Andersen 1990).

5.3. Methods

Collection and Maintenance of Animals

We collected male shore crabs from the Menai Strait, North Wales, U.K., by using baited traps. Those with a hard exoskeleton and no missing or regenerating limbs

were kept individually in holding tanks of 25 x 40 cm and 30 cm high, containing aerated sea water held at 14 ± 1 °C under continual illumination to reduce any variation in heartbeat rate due to circadian rhythms (Naylor 1996).

Subjects were fed ad libitum with mussels (*Mytilus edulis*) for at least 1 week prior to experimentation. Preliminary observations revealed that after this period, “residents” were strongly motivated to fight against “intruders” transferred from other tanks. We chose male crabs of 5–6 cm carapace width to be residents and fitted them with sensors for measuring heartbeat rate (below). Intruders were male crabs of various sizes, not fitted with sensors. We kept residents and intruders deprived of food for 24 h before each experimental session in order to standardize hunger level and associated aggressive motivation. We used crabs within 2 weeks of collection and kept them for 2 more weeks after experimentation to ensure that they were not in proecdysis (none was). Experiments were run from January to April 1999.

Monitoring Heartbeat Rate

Using cyanoacrylate glue, we fixed an infrared phototransducer (Depledge & Andersen 1990) to the carapace above the pericardial sinus. The signal from the transducer was transmitted to a computer. Heartbeat rate could either be displayed on a digital oscilloscope (Virtual Bench software) or continuously recorded by a Quick Basic program, capturing values at 0.1 s intervals and producing sequential files of voltages (see Rovero et al., 1999b for details). We used the oscilloscope to measure the heartbeat rate of residents prior to staging fights and the computer system to record data during behavioural trials. We compared the continuous heartbeat profiles with corresponding behavioural patterns. Lack of duplicate signal-processing equipment confined recording to one individual at a time. Experiments therefore were protracted and this prevented us from monitoring heartbeat rates of intruders.

Experimental Procedures and Analysis of Data

Agonistic Behaviour

We staged fights between paired opponents comprised of a resident crab (54.57 ± 0.46 , mean \pm SE ^m cm carapace width, N = 36) and an intruder of similar (55.54 ± 0.99 ^m cm, N = 18), smaller (41.97 ± 1.00 ^m cm, N = 18) or larger size (70.70 ± 1.15 ^m cm, N = 17). In the first type of pairing, the size ratio of the smaller crab to the larger was 0.85–1.00. Size

ratios within this range do not allow accurate prediction of fight outcome either in *C. maenas* (Sneddon et al. 1997a) or in other species (Huntingford et al. 1995). We used different batches of residents for evenly size-matched and unevenly matched pairings. Residents in evenly size-matched pairings were each subjected to one fight. Residents in unevenly matched pairings were each subjected to two fights, one with a larger and one with a smaller intruder, staged in random order with an interval of 2-3 days between fights. By adopting this mixed design, we compromised between the advantage of repeated measures (control of individual variation) and that of statistical independence.

We used a video camera above the aquarium to film behavioural interactions. We also directly observed behaviour through 10 cm diameter openings in black polythene sheets hung around the tank to screen subjects from disturbance. We continuously recorded the heartbeat rate of each resident for 10 min before the intruder was introduced. We then slowly introduced the intruder through an opening in the polythene sheet and released it 20-30 cm from the resident. Handling of the intruders, although careful, may have reduced readiness to fight. Any such effect, however, would have reinforced the intended rank order of aggressiveness between residents and intruders. Moreover, it should have had no differential effect regarding size of the intruder. We monitored behavioural interactions for 18 min, within which time fights were always resolved. We then removed the intruder, using the reverse procedure, and recorded the heartbeat rate of the resident for a further 18 min. Preliminary trials had shown that heartbeat rate decreased to resting levels within this time period. The single-channel input of the recording apparatus forced us to run trials sequentially.

Control

To test whether heartbeat rate of the resident could have been altered simply by the process of introducing or removing the intruder, we followed the same procedures as for the experimental treatments except that an empty hand was introduced and withdrawn from the aquarium. We randomized the order of experimental and control treatments for each crab. Residents used for size-matched pairings therefore each experienced one experimental and one control treatment, in random order. Those used for unevenly sized pairings experienced two experimental plus a control treatment,

again in random order.

Behavioural Patterns and Heartbeat Rate

Following previous studies (e.g. Smith et al. 1994; Sneddon et al. 1997b) we timed behavioural interactions from the moment the initiator adopted an aggressive display or from the moment its approach was followed by retreat of the opponent. The fight lasted until one crab, the winner, elicited the last in a series of consecutive retreats from its opponent. Usually, no further aggressive interactions occurred once the loser had made two or three retreats within about 30 s of each other. Any such re-engagements, however, were included in the measurement of fight duration.

We classified aggressive behavioural patterns as display (extended chelipeds) or contact (pushing, grasping or striking with chelipeds). Interactions could involve unilateral or bilateral acts: in the former, only one crab performed aggressive acts and the other submissively retreated, whereas in the latter both crabs reciprocally engaged in aggression. We therefore categorized interactions as follows: unilateral display (by resident or intruder), bilateral display, unilateral contact (by resident or intruder), bilateral contact.

We used chi-squared tests to compare the distributions of fight initiation and fight outcome between residents and intruders and to compare the frequencies of behavioural patterns across treatments with similarly sized, smaller and larger intruders.

We estimated “resting” heartbeat rate for each resident as the mean recorded heartbeat rate during periods of quiescence prior to the introduction of the intruder. Similarly, “fighting” heartbeat rate was taken as the mean heartbeat rate during the fight. We calculated “excess” heartbeat rate during fighting (Smith & Taylor 1993) as $((\text{fighting heartbeat rate} - \text{resting heartbeat rate}) \times \text{fight duration}) / (\text{resting heartbeat rate})$. This corresponds to the time (min) that would be required for the number of beats recorded during the period of excess to have been executed at resting level. Resting heartbeat rate did not differ significantly when crabs were paired against smaller or larger intruders (Table 2; paired t-test: $t = -0.19$, $N_1 = N_2 = 16$, NS). We therefore used the mean resting heartbeat rate of each crab to compute excess heartbeat rate. Analogous methods were used to calculate excess heartbeat rate during recovery (see results for further explanation).

We used paired t-tests or Wilcoxon's signed rank test in the case of non-normality to compare behavioural and cardiac parameters of residents when fighting smaller and larger intruders. We used t-tests for independent samples or Mann-Whitney tests to compare parameters between evenly size-matched and unevenly matched fights. To compare mean heartbeat rate between experimental and control treatments, we used paired t-tests for evenly size-matched pairings and repeated measures ANOVA for unevenly matched pairings. The same procedures were used to compare mean heartbeat rate after removal of the intruder with that during the control treatment.

Respirometry

Oxygen consumption rate was measured using closed respirometers of approximately 300 ml in volume. We removed small samples of water from the respirometers to measure oxygen partial pressure (below). To determine the relationship between heartbeat rate and respiration rate, we measured both variables in crabs initially disturbed by handling and again during recovery. We took crabs fitted with phototransducers and held them below the water surface for 2 min, allowing the legs to move freely. This procedure disturbed the crabs sufficiently to cause an elevation in heartbeat rate. We immediately placed each crab in a respirometer and recorded oxygen uptake and heartbeat rate over a period of 10 min. We then opened the respirometer to the surrounding seawater to regain ambient oxygen tension and allowed the crabs to settle for 2 h, during which time heartbeat rate returned to resting levels. We resealed the respirometers and measured oxygen uptake over a further 10 min, while crabs were still in an undisturbed state.

Oxygen partial pressure was measured with an oxygen electrode (Radiometer E5047) held in a cuvette maintained at 14°C and connected to an oxygen meter (Strathkelvin Instruments). Oxygen uptake rates were calculated according to Dejours (1981), using oxygen solubility coefficients corrected for temperature and salinity. The resultant data were plotted against corresponding changes in heartbeat rate. We used linear regression to analyse the relationship between heartbeat rate and oxygen consumption rate.

All statistical tests used two-tailed probability criteria at $\alpha=0.05$.

Ethical note

On local shores, *C. maenas* forage actively in daylight (Cunningham 1983) and continual illumination should not have caused undue stress while crabs were kept in the laboratory. Aggression did not escalate during fights and opponents never inflicted injury upon one another. Following experimentation, all crabs were returned to the collection site. We believe that, in comparison with other methods, the technique used in this study minimizes the potential stress to subjects. Fitting the phototransducer involved removing the crab from the tank, cleaning the carapace and waiting 1-2 min for the glue to dry. The behavioural performance of crabs did not appear to be altered by the presence of sensors and wires, whose weight of 1.2 g represented about 3 % of total body mass. The transducer was cubic, of width 0.5 cm, fitted to a wire of gauge 0.5 mm. The loosely suspended wire appeared not to impede movement of the crab.

5.4. Results*Behavioural Strategies*

Forty-eight fights were recorded. Resident crabs initiated fights significantly more frequently than intruders (Table 1; chi-square: $\chi^2_1=14.08$, $P<0.01$). The proportion of initiations by residents decreased from 89 % when fighting smaller intruders to 64 % when fighting larger intruders. Chance of winning depended primarily on size of the opponent: in unevenly matched fights, the larger crab always won, regardless of residence status. When evenly size-matched, residents won 69 % of fights, but probably owing to small sample size, this was not significantly different from random expectation (Table 1, $\chi^2_1=1.17$, $P=0.28$).

Most fights were resolved by unilateral or bilateral contact by the resident, with a minor proportion resolved by unilateral display by the resident, usually against smaller intruders (Fig. 1). Only one fight, between evenly size-matched crabs, was resolved by bilateral display and only one was resolved by unilateral contact, performed by the larger intruder. Although fights involving bilateral contact (grasps and strikes) were more likely to start by unilateral contact, in other cases intense bilateral contacts were performed without any previous displaying. There was, therefore, no apparent escalation in the intensity of aggression. The distribution of behavioural patterns was significantly different across the three types of pairings (chi-

square test: $\chi^2_2=13.30$, $P<0.02$), bilateral contact being the dominant pattern both in size-matched pairings and in those with larger intruders. Consequently, different behavioural patterns were associated with different outcomes. When the resident won, unilateral contact occurred more frequently than bilateral contact, but the reverse was true when the resident lost ($\chi^2_2=9.94$, $P<0.01$). Fights involving bilateral contact were the most intense, but did not result in injury.

Table 1. Number of interactions between resident crabs and intruders of smaller, similar and larger size, respectively. Interactions are further classified according to initiation and outcome of fights. The initiator is the crab who first performed aggressive behaviour or whose approach was followed by the retreat of the other crab. The winner is the crab who elicited a series of consecutive retreats from the other crab.

	Relative size of intruder		
	Smaller	Similar	Larger
Resident initiated	16	12	9
Intruder initiated	2	4	5
Resident won	18	11	0
Intruder won	0	5	14

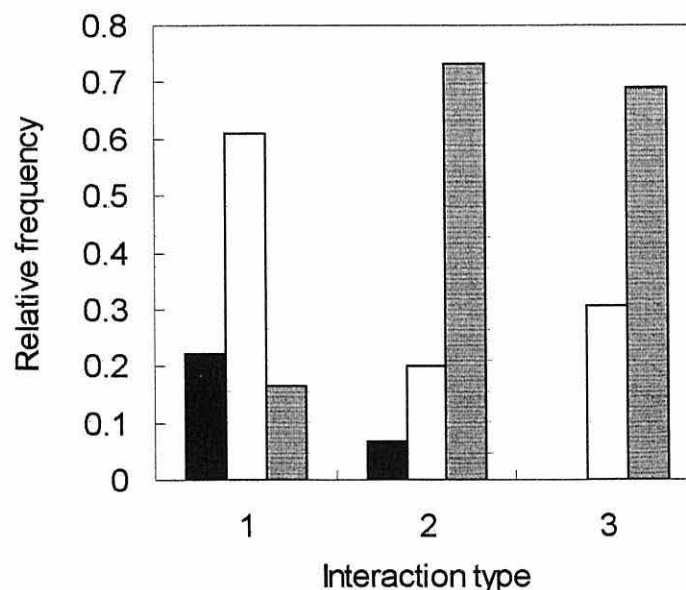


Figure 1. Proportional frequencies of different behavioural acts during fights between resident crabs and intruders. Grouped along the abscissa are fights with (1) smaller, (2) similar and (3) larger intruders, respectively. Black bars = unilateral display by the resident, white bars = unilateral contact by the resident, grey bars = bilateral contact.

In most interactions between evenly size-matched crabs and between residents and larger intruders, fighting progressed as an almost continuous bout of aggression. Seven out of eighteen fights against smaller intruders consisted of two to three bouts, in which the resident pursued the intruder around the aquarium. Fight duration was weakly correlated with the size ratio of opponents (Fig. 2; Pearson correlation: $r=0.32$, $P<0.02$). Fight duration increased from 2.40 ± 0.76 min (mean \pm SE) for fights between residents and smaller intruders, to 4.51 ± 0.76 min for fights between evenly size-matched opponents (Table 3; t-test: $t = -2.47$, $N_1=17$, $N_2=16$, $P<0.03$, marginally nonsignificant after Bonferroni correction). Fight duration between residents and larger intruders (3.28 ± 0.53 min) was not significantly different from those of the other types of pairings. Moreover, duration of fights won and lost by residents were not significantly different either for unevenly size-matched pairings (above) or for evenly size-matched pairings (4.89 ± 1.07 min and 3.68 ± 0.70 min, resident won and lost, respectively; t-test: $t=0.73$, $N_1=11$, $N_2=5$, $P=0.48$).

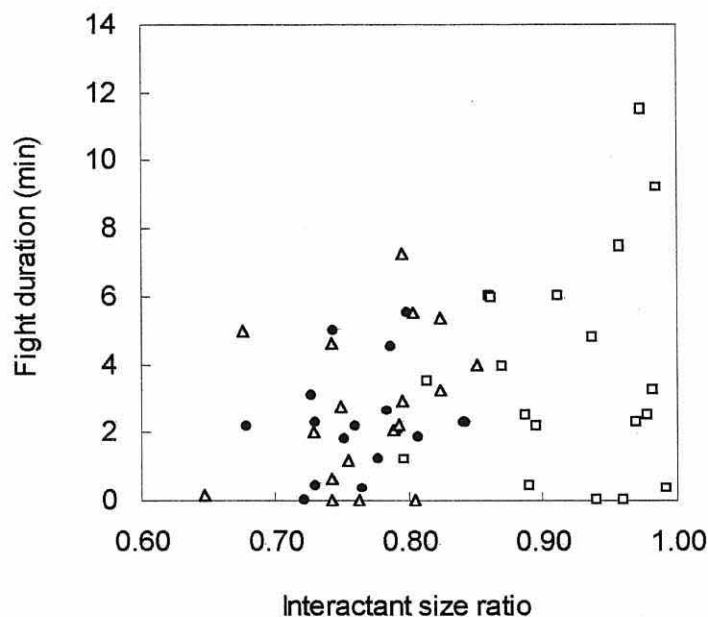


Figure 2. Duration of aggressive interactions between resident crabs and intruders, plotted according to the ratio between the carapace width of the smaller crab to that of the larger. Data are grouped according to whether the intruder crab was smaller (closed circles), similar (open squares) or larger (open triangles) than the resident crab.

*Heartbeat rate and respiration rate*Variation in heartbeat rate

Heartbeat profiles are exemplified in Fig. 3. Resting heartbeat beats were arrhythmic, with frequent periods of pausing resulting in highly variable heartbeat rates ranging from 20-60 beats min^{-1} (Fig. 3 & Fig. 4). At the moment the intruder was introduced, heartbeat rate of the resident crab generally paused for about 10 sec, before increasing rapidly to values between 80-120 beats min^{-1} . Subsequently, the highest heartbeat rates were recorded both during fighting and during the immediate post-fighting stage, although the initial bout of aggression was often associated with an immediate decrease in heartbeat rate (Fig. 3).

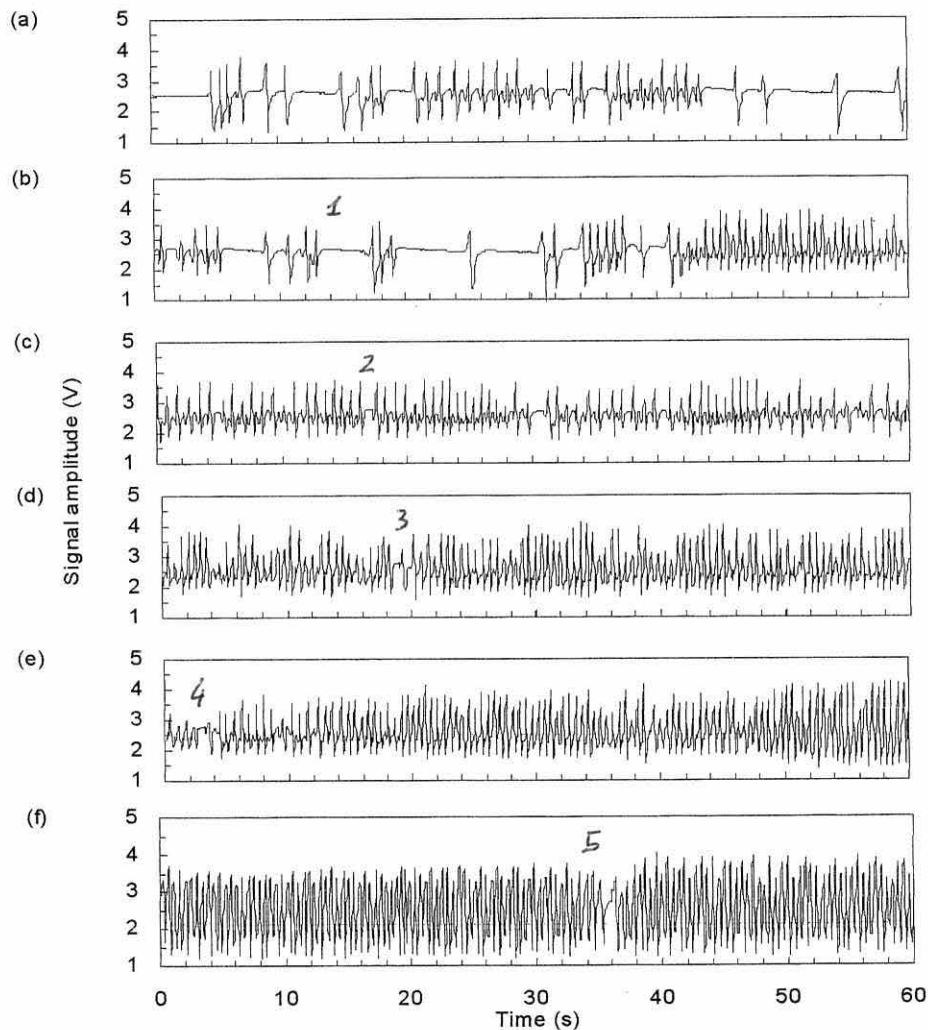


Figure 3. Continuous recording of mean heartbeat rate during successive phases of a fight between a resident and similarly sized intruder. (a) undisturbed condition; (b) introduction of the intruder; (c)-(f) fighting bout. 1 = point of introduction of intruder, 2 = initiation of fighting by the resident, followed by unilateral display and contact, 3 = retreat of the intruder, 4 = re-engagement by the resident and phase of bilateral contact, 5 = retreat by the intruder. This fight was won by the resident.

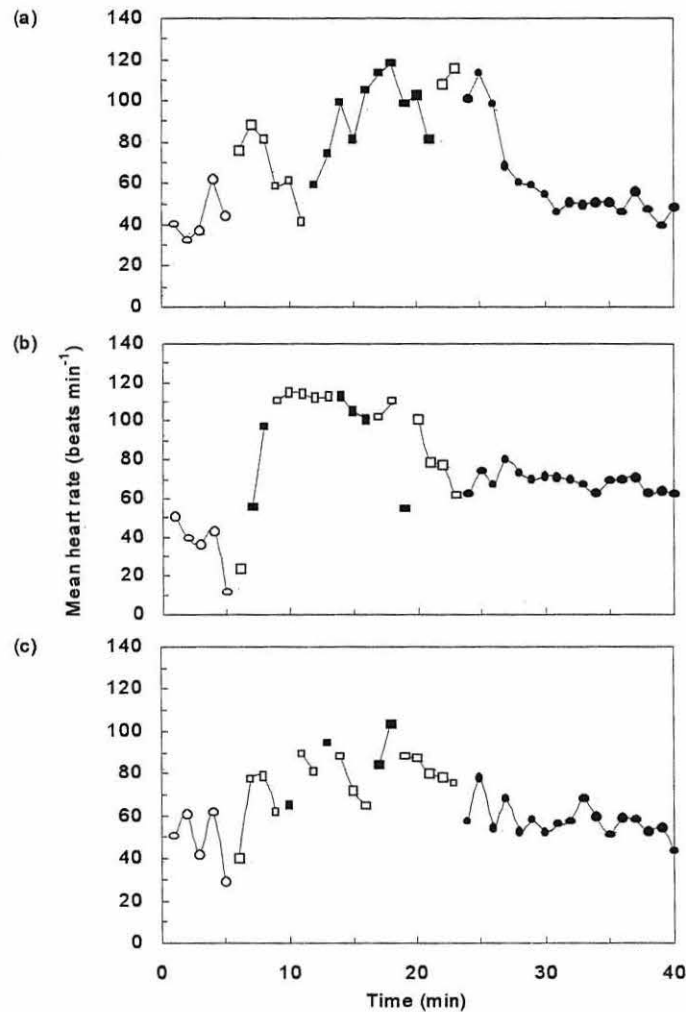


Figure 4. Profiles of heartbeat rate during successive phases of fights between a resident crab and intruders of different relative size: (a) smaller, (b) same and (c) larger. Open circles = undisturbed conditions, before introduction of the intruder; squares = presence of the intruder, divided into periods of fighting (black squares) and non-fighting (open squares); closed circles = period after removal of the intruder.

General trends, revealed by pooling data among residents, are shown in Fig 5. Heartbeat rate reached maximum values within 2-3 min of the appearance of the intruder, corresponding to the initiation of fighting. Heartbeat rates remained elevated for various lengths of time, broadly reflecting differences in fight duration. This temporal variation was considered in the calculation of excess heartbeat rate (below). Heartbeat rate decreased after the resolution of fights, slightly increasing again during 1-2 min after removal of the intruder (see below for analysis of the recovery phase). Mean heartbeat rate profiles did not significantly vary between crabs that fought against intruders of similar, smaller and larger sizes (Fig. 5, Table 2). Therefore, since fights against smaller and larger intruders had opposite outcomes, mean heartbeat rate

did not differ between winners and losers. Mean heartbeat rate did not vary consistently between resident crabs fighting by bilateral contacts (87.82 ± 3.11 , mean \pm SE, $N=21$), unilateral contacts (93.43 ± 3.84 , $N=15$), or unilateral displays (84.60 ± 9.07 , $N=5$).

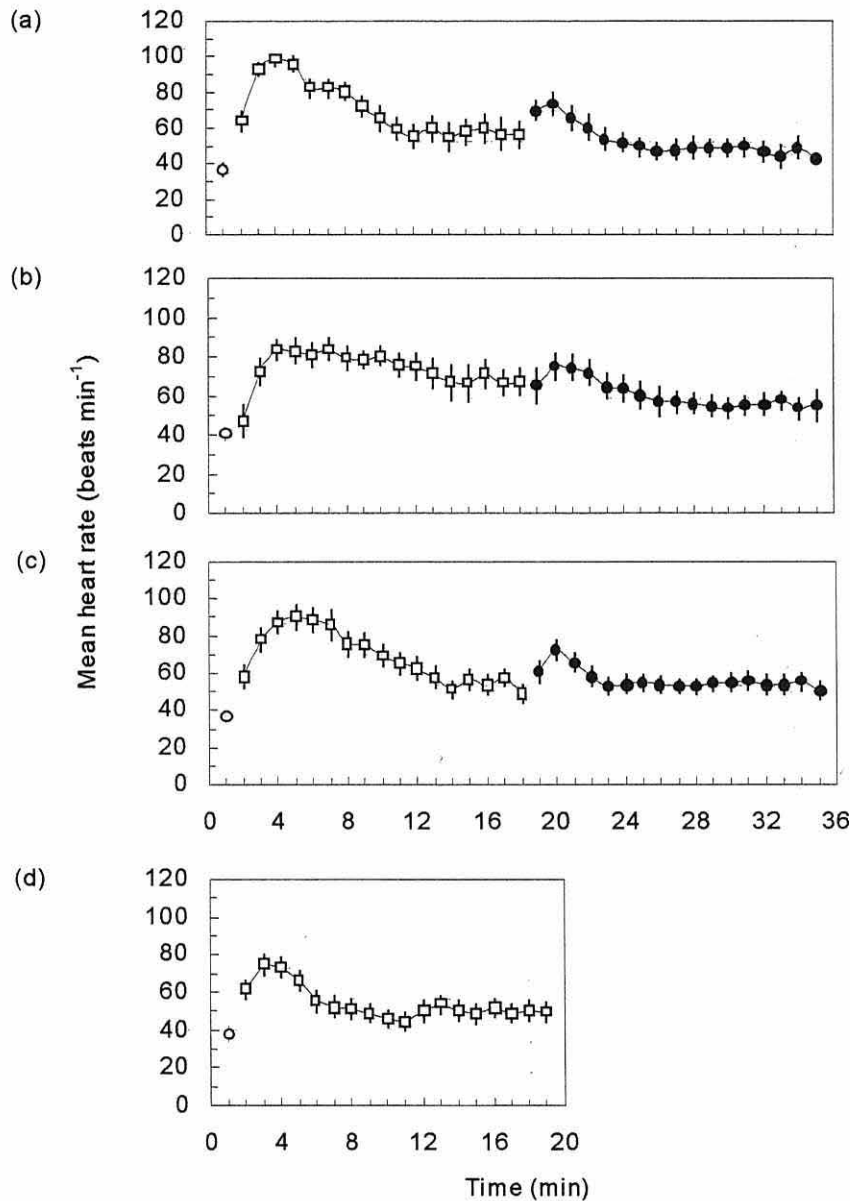


Figure 5. Temporal profiles of mean heartbeat rate (\pm SE), each obtained by pooling data for different resident crabs, during successive phases of fighting with intruders of different relative size: (a) smaller, (b) similar and (c) larger size, respectively. The same resident crabs were used for fights (a) and (c). Open circle = mean heartbeat rate during 10 min prior to the introduction of the intruder in the tank; open squares = period during which the intruder was inside the tank; closed circles = period from removal of the intruder. Profile (d) records the mean heartbeat rate, pooled for all crabs, during undisturbed conditions (open circle) and control treatment (open squares).

Heartbeat rate of resident crabs during fighting was significantly higher than during control treatments (Table 2; evenly size-matched opponents, paired t-test: $t=8.23$, $N_1=N_2=16$, $P<0.001$; unevenly matched opponents, repeated measure ANOVA, $F_{2,24}=18.52$, $P<0.001$). In contrast, mean heartbeat rate after removal of the intruder was not significantly different from control values (Table 2; evenly size-matched opponents: $t=1.95$, $N_1=N_2=16$, NS; unevenly matched opponents: $F_{2,30}=0.86$, NS).

Table 2. Heartbeat rate (beats min^{-1}) of resident crabs during different phases of the fight against intruders of similar, smaller and larger size, respectively. 'Resting' refers to 10 min prior to the introduction of the intruder. 'Intruder in tank' refers to the total period of 18 min during which the intruder was in the tank along with the resident. 'Fighting' is the phase of aggressive interaction. 'Intruder removed' refers to the period of 18 min after removal of the intruder. 'Control' refers to the period of 18 min after disturbance of the resident by the introduction of the observer's hand. Note that the same crabs were used for fights against smaller and larger intruders, whereas different crabs were used for fights against intruders of the same size. Data are means \pm SE; sample sizes are in parentheses.

	Similar	Smaller	Larger
Resting	39.49 ± 3.18 (16)	36.87 ± 4.34 (16)	37.96 ± 4.90 (16)
Intruder in tank	72.85 ± 4.85 (16)	74.95 ± 4.88 (16)	73.85 ± 5.40 (16)
Fighting	86.43 ± 4.45 (16)	91.22 ± 4.00 (14)	91.45 ± 5.28 (13)
Intruder removed	59.20 ± 6.00 (16)	59.06 ± 6.53 (16)	58.57 ± 5.96 (16)
Control	50.50 ± 3.57 (16)	51.96 ± 6.01 (16)	

Excess heartbeat rate

Excess heartbeat rate during fights was not significantly different between pairings with smaller and larger intruders (Table 3; Wilcoxon signed rank test: $Z=-0.62$, $N_1=N_2=11$, NS). Although mean excess heartbeat rate was higher for crabs fighting against evenly size-matched opponents than against smaller or larger opponents (Table 3), the differences were not statistically significant (Mann-Whitney test: smaller intruders $U=207.0$, $N_1=16$, $N_2=14$, NS, larger intruders $U=174.0$, $N_1=16$, $N_2=13$, NS). Excess heartbeat rate during fighting was significantly correlated with fight duration both for winners and for losers, as expected since the former parameter is not independent of the latter (Pearson correlation: $r=0.81$, $df=23$, $P<0.001$ for winners, and $r=0.70$, $df=15$, $P<0.003$ for losers).

After fighting, heartbeat rate generally declined smoothly to resting levels (Fig.

5). Removal of the intruder, however, slightly raised the heartbeat rate for 3-4 min, similar to the effect of the control treatment. Consequently, recordings during the first 4 min following removal of the intruder were omitted from data analysis. Lines of best fit were obtained by linear regression after logarithmic transformation of time. By extrapolating from these regressions, we predicted the time that would be taken for heartbeat rate to return to resting levels, as a measure of recovery time from fighting.

Table 3. Cardiac and respiratory parameters of resident crabs fighting against intruders of similar, smaller and larger size, respectively. HR=heartbeat rate; see text for explanation of parameters. Data are means \pm SE; sample sizes are in parentheses.

	Smaller	Similar	Larger
Fight duration (min)	2.40 \pm 0.38 (14)	4.51 \pm 0.76 (16)	3.28 \pm 0.53 (13)
Excess HR during fighting (min)	4.4 \pm 1.1 (14)	6.6 \pm 1.6 (16)	5.3 \pm 1.2 (13)
Excess O ₂ demand during Fighting (μ mol O ₂)	2.38 \pm 0.52 (14)	3.63 \pm 0.80 (16)	3.35 \pm 0.66 (13)
Predicted recovery time (min)	29.36 \pm 5.17 (11)	42.1 \pm 10.3 (14)	62.0 \pm 12.2 (10)
Excess HR during Recovery (min)	29.6 \pm 12.7 (11)	28.8 \pm 9.4 (14)	37.90 \pm 11.3 (10)
Excess O ₂ demand during recovery (μ mol O ₂)	9.83 \pm 2.89 (11)	13.23 \pm 4.11 (14)	16.49 \pm 4.27 (10)
Total excess HR (min)	34.9 \pm 12.9 (11)	34.7 \pm 10.2 (14)	43.8 \pm 11.1 (10)
Total excess O ₂ demand (μ mol O ₂)	12.47 \pm 3.05 (11)	16.72 \pm 4.51 (14)	19.75 \pm 4.11 (10)

Predicted recovery time was highly variable, although for 20 out of 36 crabs with significant regressions, recovery was completed within 30 min of fight resolution. Mean predicted recovery time was ranked in the order: pairings with smaller intruder < evenly size-matched pairings < pairings with larger intruder (Table 3), but pairwise comparisons were not statistically significant. From data pooled across pairing categories, predicted recovery time of residents was 41.4 \pm 14.6 min (mean \pm SE) for winners and 64.9 \pm 14.6 min for losers. Excess heartbeat rate during recovery, computed by integrating the recovery function over the predicted recovery time, did not significantly vary with size of the intruder (Table 3) and was not significantly correlated with fight duration, either for winners (Pearson correlation: $r=0.08$, $df=18$,

NS) or for losers ($r=0.31$, $df=10$, NS).

Total excess heartbeat rate was estimated as the sum of excess heartbeat rate during fighting and recovery and therefore increased with the relative size of opponents (Table 3), although pairwise comparisons were not statistically significant (repeated measures ANOVA: $F_{1,8}=3.57$, $P=0.10$, observed power = 0.38). Again, this parameter was not significantly correlated with fight duration, either for winners ($r=0.19$, $df=18$, NS) or for losers ($r=0.37$, $df=10$, NS).

Metabolic demand

Heartbeat rate was linearly related to oxygen consumption rate (Fig. 6). The regression: O_2 consumption ($\mu\text{mol } O_2 \text{ min}^{-1} \text{ g}^{-1}$) = $0.00555 + 0.00035 \times \text{heartbeat rate}$, $F_{1,24}=21.66$, $R^2=0.47$, $P<0.001$, was used to predict oxygen demand from excess heartbeat rate during fighting and recovery (Table 3). The sum of these values gave the total excess oxygen demand resulting from fighting. Mean total excess oxygen demand was weakly associated with the size of the opponent (Table 3), the difference between fights with smaller and larger contenders being marginally non-significant prior to Bonferroni correction (paired t-test: $t=-2.18$, $N_1=N_2=9$, $P=0.06$).

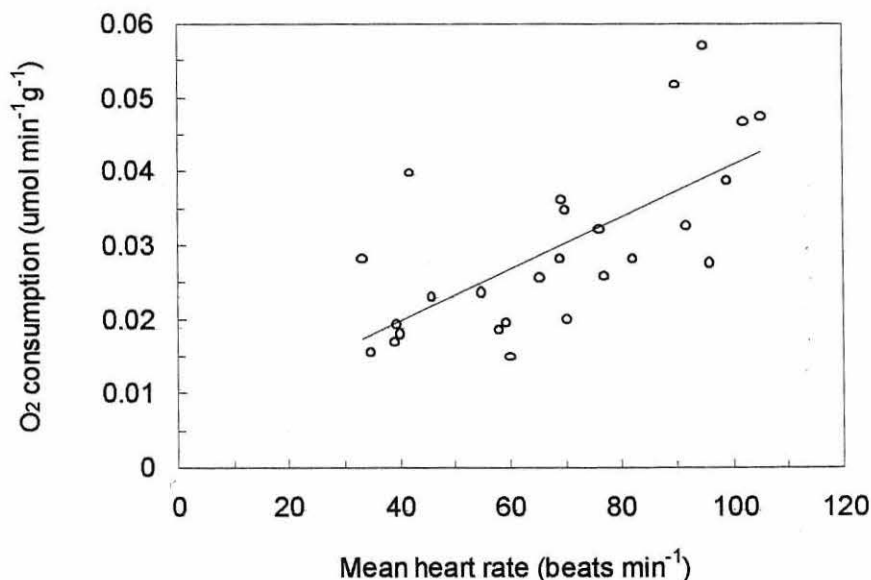


Figure 6. Relationship between oxygen consumption and heartbeat rate of crabs monitored before and after 2 h of exercise.

5.5. Discussion

Behavioural Strategies

As intended, the two experimentally controlled asymmetries between opponents produced fights differing in duration and intensity. Residents were slightly more likely to initiate fights than intruders and the relative size of opponents clearly influenced fight duration and behavioural content. Fights tended to be longer and bilateral contacts more frequent when opponents were closely matched in size, as reported for other crabs (e.g. Glass & Huntingford 1988; Smith et al. 1994). Compared with fights against evenly sized opponents, duration and intensity were reduced more in fights against smaller than against larger intruders. This asymmetry reflected greater persistence of the resident, rather than aggressiveness of the larger intruder. Fighting therefore was intensified when the risk of losing ownership was increased by greater size of the opponent. Having obtained fights differing in quality, we could then examine any associated differences in metabolic cost.

Energetic costs of fighting

Elevated heartbeat rate associated with aggressive behaviour was maintained throughout fights. Estimated energetic cost during fighting therefore increased with fight duration, as reported for other crabs (Smith & Taylor 1993) and for insects such as the house cricket (Hack 1997). Contrary to expectation, however, the elevation in heartbeat rate did not vary according to the type of fighting behaviour adopted. Moreover, although the longer and more intense fights between evenly size-matched opponents were associated with higher excess heartbeat rate, this was compensated by a shorter recovery time compared with fights involving larger opponents. As a result, the total energetic cost was not significantly different in the two cases. Similarly, Smith & Taylor (1993) found that the energetic cost of fighting was not correlated with the ordinal intensity of fights, even though more intense interactions involved higher rates of oxygen consumption. These authors suggest that the relationship between fight characteristics and energetic cost is likely to be complicated by the permutations of different behavioural acts that may be involved. This is supported by our data, which represent a greater relative size range of opponents and associated variety of behavioural content and fight duration. Nevertheless, the present

data must be interpreted cautiously since the regression of oxygen consumption rate on heartbeat rate (Fig. 6) accounted for only 47% of the variance, perhaps weakening the power of our analysis to detect small differences in energetic cost associated with fighting intensity.

As the average elevation in heartbeat rate during the relatively short period monitored after fighting did not differ significantly from that prior to fighting, it is reasonable to assume that fighting is not an energetically expensive activity. This supports previous reports that fighting is much less expensive than enforced exercise (Sneddon et al. 1999) and has no adverse effect on subsequent activities (Thorpe et al. 1995). We found, however, that resident crabs having fought against larger intruders took longer to recover than when they had fought against smaller intruders. This result cannot adequately be explained by the intensity and duration of fighting, which were highest for evenly size-matched contests. Instead, it is more likely that metabolic cost to the resident is ultimately determined by perceived risks of losing ownership or of injury, both of which are likely to increase with size of the opponent and may engender a prolonged state of alertness (see Austad 1983). Field observations corroborate this argument. Cunningham (1983) filmed shore crabs, unconstrained on the flooding tide, competing for access to a patch of mussels. Mean residence time was longer for larger crabs, who frequently used physical contact to fight intruders of similar size and elicited retreat from smaller crabs by using less intense aggressive activity without physical contact. Smaller crabs fed in an opportunistic manner, quickly visiting the patch and then immediately retreating. The prolonged alerted state of smaller losers, shown in the present study, therefore might be an adaptive response anticipating further engagement with superior opponents. An analogous, presumably adaptive state of alertness has been revealed by heartbeat recordings in mussels threatened by risk of predation (Rovero et al. 1999b).

In conclusion, the noninvasive, externally mounted infrared phototransducer provided a satisfactory method of measuring the heartbeat rate of crabs, which itself was a good indicator of their aerobic metabolic rate. Metabolic cost was weakly determined by duration of the fight and appeared to be largely independent of its behavioural content or intensity (c.f. Hack 1997, but see also Smith & Taylor 1993). Moreover, greater size of the intruding opponent was accompanied by prolonged recovery and therefore greater total energy cost of fighting (Table 3), independently

of fight duration or intensity. Although tentative, owing to limited power of the statistical test, this result contradicts the game-theoretical prediction that costs should be higher when contests are more evenly matched (e.g. Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Enquist & Leimar 1983). On the other hand, the prolonged elevation of metabolic rate may adaptively prepare the individual for further engagement if larger size of the intruder indicates risk of injury and/or loss of ownership. Another, complementary, interpretation is that residents fought harder against larger intruders because they perceived that greater effort was required to successfully defend the resource. Supporting evidence is confined to the weak correlation between fight duration and size of the intruder.

Crabs are proving to be excellent models for testing theories on fighting behaviour and this should be enhanced by measuring the energetic costs of alternative behaviours under a wider range of asymmetries and risks. For such purpose, noninvasive monitoring of heartbeat rate will be of great advantage.

Chapter 6. When time is of the essence: choosing a currency for prey-handling costs

6.1. Abstract

- 1) Heartbeat rate was measured in shore crabs, *Carcinus maenas*, feeding on mussels, *Mytilus edulis*, in order to estimate the energetic cost of handling prey and so to assess the relative importance of energy and time as costing currencies.
- 2) Energetic handling costs represented only about 2% of corresponding gains.
- 3) The tendency of profitability (gain per unit handling time) to increase with prey size was weakened by including energetic handling costs.
- 4) Time was judged to be a more appropriate currency than energy for costing prey-handling behaviour.
- 5) The importance of time as a costing currency, either through the principle of lost opportunity or through exposure to mortality risk, may extend to other behavioural systems, including aggression.

6.2. Introduction

Most biological objectives are gained at some cost, resulting in dichotomies at all levels, from the evolution of specialist versus generalist genotypes (Baker 1965), physiological trade offs (Sibly & Calow 1986), to alternative behavioural decisions (Krebs & Davies 1991). Theoretical consideration of physiological and behavioural levels usually assumes that fitness is positively correlated with the maximization of gain to cost, measured in some appropriate currency (Krebs & Kacelnik 1991). Recognising the appropriate currency for costing, however, may not be straightforward when there are plausible alternatives. This is generally the case with foraging behaviour, where a behavioural decision incurs costs in metabolic energy and time, specific to the chosen action. Time itself may represent lost opportunity to perform other acts (Stephens & Krebs 1986) or exposure to mortality risk (Sih 1993). Different types of cost could, in principle, be levied in different proportions depending on context. Predicting this context has led to the concepts of energy maximizing, time minimizing and risk minimizing behaviour (Stephens & Krebs 1986; Hughes 1993).

Energetic costs, however, tend to be ignored in experimental investigations simply because it is technically difficult to measure metabolic rate associated with specific behavioural acts. For example, Elner & Hughes (1978) used prey-handling time as the only costing currency in their experiments with shore crabs, *Carcinus maenas* (L.), feeding on mussels, *Mytilus edulis* L., designed to test predictions of optimal diet theory (Charnov 1976). This theory proposes that prey selection should partially depend on profitability, defined as the net energy gained per unit handling time per prey item and therefore including the energetic cost of handling the prey. By using heartbeat rate to indirectly measure metabolic rate of the forager, the present study examines the relative importance of energy and time as costing currencies for the crab-mussel system.

6.3. Methods

Experimental animals

Shore crabs were collected from the Menai Strait, North Wales, U.K., by using baited traps. Following Elner & Hughes (1978), individuals chosen for experiments were 5-6 cm in carapace width (56.4 ± 2.5 ^mcm, mean \pm standard error, $n = 20$) and were all males, in order to avoid any potential bias caused by sexual differences in morphology and prey-handling behaviour. All chosen individuals possessed a hard exoskeleton with no missing or regenerating limbs. They were kept individually in holding tanks of 25 x 40 x 30 cm, containing aerated sea water held at 15 ± 1 °C, and under continual illumination to avoid circadian rhythms in behaviour (Naylor 1996). Crabs were fitted with sensors for monitoring heartbeat (below). Common mussels, *Mytilus edulis* L., were collected from a moderately sheltered shore within 2 miles of the Menai Strait. Three non-overlapping size classes, based on shell length and known to be within the range readily accepted by crabs of 5-6 cm in carapace width (Elner & Hughes 1978), were chosen as prey: small, 13-16 mm; medium, 20-23 mm; large, 27-30 mm.

Over a period of 3-4 days before experimental trials began, each crab was allowed to consume 8 mussels from each size class. This would help to standardise prey-handling skills among crabs, since learned improvement of skills is complete after feeding on a sequence of 5 to 10 prey items (Cunningham & Hughes 1984). Crabs were deprived of food for 24 h before each experimental trial in order to standardise

hunger level and associated predatory motivation. After training and between experimental trials, crabs were fed on extracted mussel flesh, so avoiding potential alteration of shell-breaking skills.

Crabs were used within 2 wk of collection and kept for a further 2 wk after experimentation to ensure that they were not in proecdysis, which could have affected foraging behaviour (none was). All crabs were released at the collection site, together with any mussels not used as prey. Experiments were run from April to June 1999.

Monitoring heartbeat rate

Heartbeat rate was monitored by an infrared phototransducer (Depledge & Andersen 1990) fixed to the carapace and connected by wires to a digital oscilloscope and PC (for details see Rovero et al. 1999b). We used the oscilloscope to measure the heartbeat rate of crabs in resting condition prior to the beginning of each trial and the PC to continually record data during trials. Heartbeat profiles recorded on the PC were compared with corresponding video recordings of behaviour.

Experimental procedures and analysis of data

Prey-handling trials

A video camera was used to film prey-handling behaviour. Animals were also observed through 10 x 5 cm openings in black polythene sheets hung around the tanks to screen subjects from disturbance. Heartbeat rate was continuously recorded for 5 min before placing a mussel about 5 cm in front of the crab. To minimise disturbance, the prey was gently lowered on a support, 50 cm long and made of transparent PVC with a distal ledge to carry the mussel. The mussel was contacted by the crabs within 5 to 60 s and heartbeat rate was continuously recorded during the ensuing prey-handling behaviour. Following Hughes & Elner (1978), the following events were timed by direct observation: (i) breaking time: the time elapsing from the first contact with the prey to the first bite of exposed flesh, (ii) eating time: the period from the end of breaking time to the abandonment of the empty shell, (iii) handling time: the sum of breaking and eating time.

Heartbeat rate was recorded continuously during prey-handling behaviour and then for 1 min periods, taken at 5 min intervals, for a further 80 min after the crab had finished handling the mussel. Mean heartbeat rate prior to the introduction of the

mussel was defined as “resting” heartbeat rate. “Breaking” and “eating” heartbeat rates were taken as the mean rates during the corresponding behavioural phases, while “handling” heartbeat rate was taken as the grand mean rate over the entire prey-handling process. Once crabs had finished handling their prey, heartbeat rate declined slowly toward resting levels, but did not reach these levels within the 80 min observation period. Regression analysis of individual recovery trajectories was used to predict the time that would be taken for heartbeat rate to return to resting level. Logarithmic transformation of time generally gave the lines of best fit. The regression equations were then used to predict the time required to recover from the exertion of handling the prey. The above procedures were repeated using a mussel from each size class in random order, allowing 2-3 days between trials.

Energetic parameters

Heartbeat rate (beats min^{-1}) was converted to specific respiration rate ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ min}^{-1}$) by using the linear relationship between cardiac and respiratory rates previously established for similarly sized male crabs (Rovero et al. 1999c). Prey-handling cost, representing the metabolic demand of exertion, was based on the difference between handling heartbeat rate and resting heartbeat rate. As resting heartbeat rate did not differ significantly among trials with small, medium or large mussels (Fig. 1), the mean value per crab was used to compute excess heartbeat rate. Respiration rates were corrected for individual body mass, ignoring allometry since individual variation in mass was slight ($\text{CV} = 4.4 \%$), then multiplied by the duration of corresponding components of handling behaviour to yield respiratory demand. Excess respiratory demand during recovery was estimated similarly, but required integration of the declining heartbeat rate trajectories. Respiratory demand was converted to energy expenditure by applying a mean oxyenthalpic equivalent of $484 \text{ kJ mol}^{-1} \text{ O}_2$ (Gnaiger 1983).

Energy gained from the prey was estimated from mussel shell length by applying the allometric function given by Elner & Hughes (1978) for relating flesh content to shell length. The slight inflation of estimated energetic gain resulting from incomplete ingestion (traces of adductor muscle are left attached to the shell) and assimilation (assimilation efficiency of carnivorous arthropods is likely to exceed 90% (Pavlyutin 1970)) would be similar for all prey. The ratio of energetic gain to cost was

computed both for handling cost alone and for handling plus recovery costs.

Profitability was calculated both as the gross gain per unit handling time (energy ingested / handling time) and as the net gain per unit handling time ((energy consumed – energy expended on handling prey) / handling time).

Statistical analysis

Mean values of behavioural durations and heartbeat rates were compared across prey sizes by repeated measures ANOVA, using the Huynh-Feldt epsilon to reduce degrees of freedom in compensation for non-sphericity of the covariance matrix of transformed variables (Norušis 1993).

6.4. Results

Examples of heartbeat profiles are shown in Fig. 1. Resting heartbeat rate was arrhythmic with frequent pauses, generating values of 30-60 beats min^{-1} (Figs. 1 and 2).

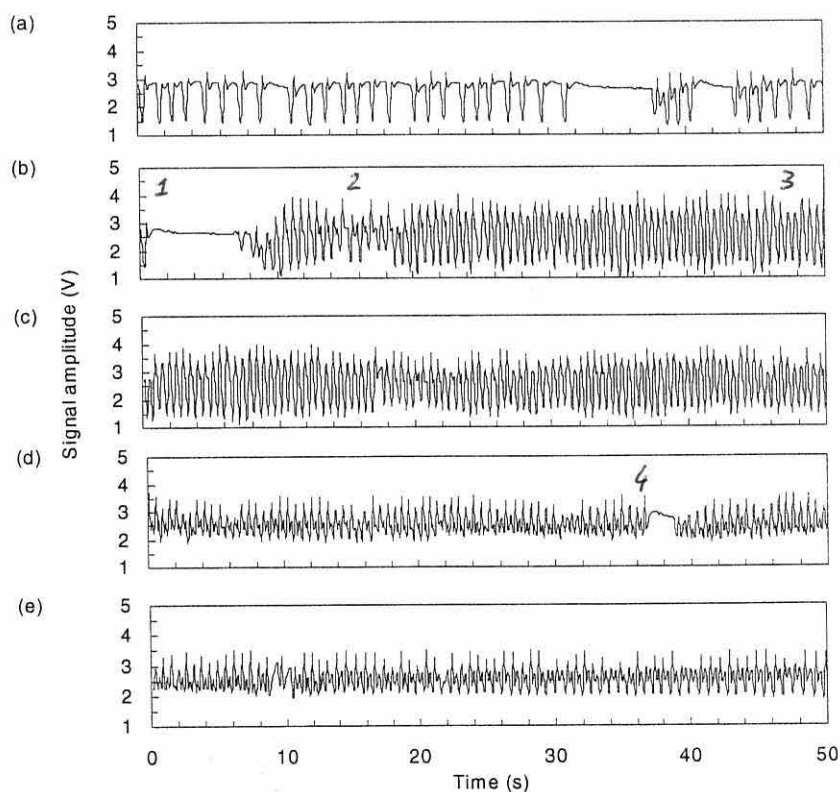


Figure 1. Continuous recording of heartbeat of a crab during successive phases of prey-handling of a small (13-16 mm) mussel: (a) undisturbed condition; (b)-(d) prey-handling, 1 = point of introduction of the mussel, 2 = contact and beginning of breaking time, 3 = beginning of eating time, 4 = abandonment of the empty shell and beginning of recovery; (e) recovery.

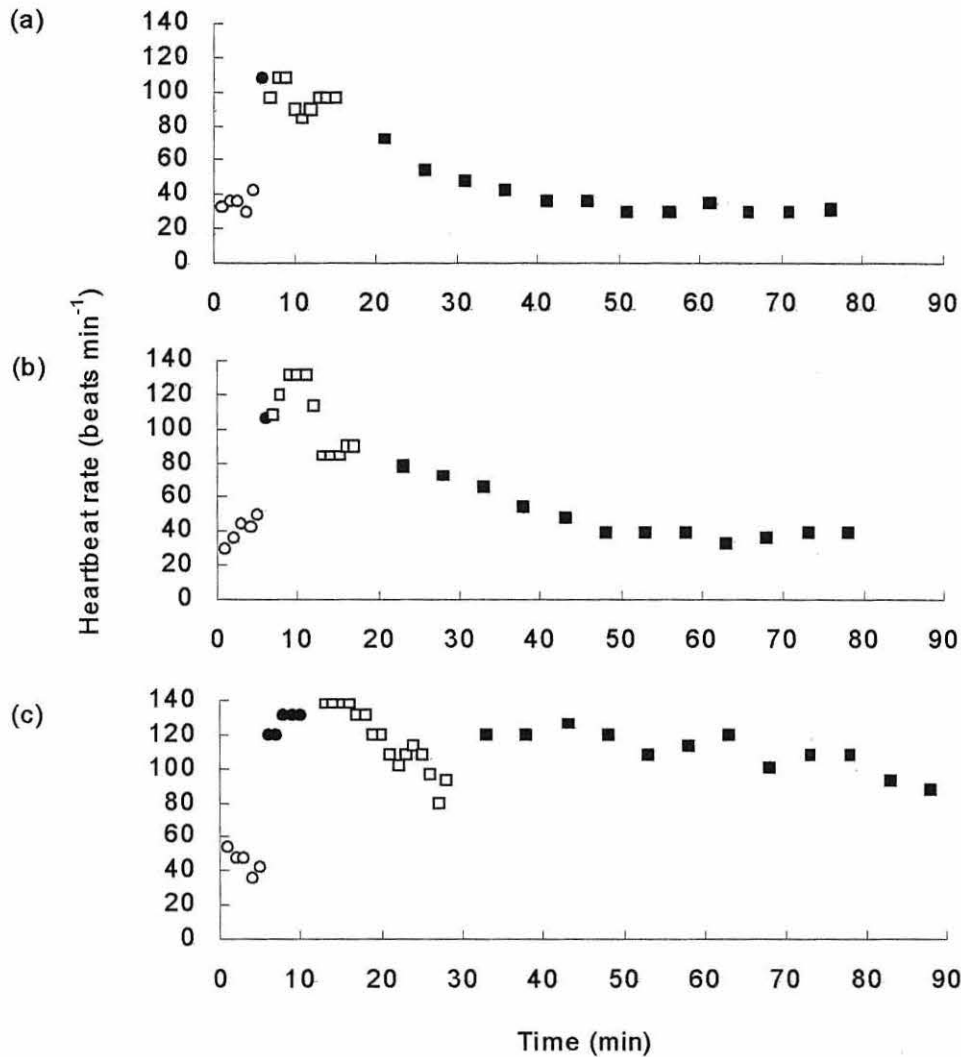


Figure 2. Profiles of mean heartbeat rate of a crab during successive phases of feeding on three mussels of different size classes: (a) small, (b) medium and (c) large. Open circles = undisturbed conditions, before introduction of the mussel; closed circles = breaking time; open squares = eating time; closed squares = recovery.

After introduction of the mussel, heartbeat rate increased as the crab moved towards the prey. This brief, transitory phase was excluded from further consideration. Breaking heartbeat rate ranged from 90 to 130 beats min⁻¹, mean values increasing significantly with prey size (Fig. 3a, Table 1). Heartbeat rate increased further during the eating phase for small and medium prey but remained similar to breaking levels for large prey, this difference being marginally non-significant (Fig. 2, Table 1). Predicted recovery time tended to increase with prey size but was highly variable and the trend was not statistically significant (Table 1 and 2).

Table 1. Duration of behavioural acts and associated heartbeat rate (HR) of crabs feeding on mussels. "Resting" refers to 5 min prior to the introduction of the prey into the aquaria. Data are means with standard errors. Sample sizes for measurements of recovery time are in parentheses.

	Mussel shell length (mm)			F	df	P
	13-16	20-23	27-30			
Sample size	17 (14)	17 (14)	15 (12)			
Breaking time (s)	32.9 ± 4.0	119.1 ± 28.5	624.6 ± 172.6	10.18	1,15	< 0.006
Eating time (s)	295.6 ± 34.8	614.5 ± 81.3	948.1 ± 139.2	16.35	2,28	< 0.001
Handling time (s)	328.5 ± 36.8	733.6 ± 96.0	1573 ± 225	21.98	1,19	< 0.001
Recovery time (min)	83.1 ± 14.7	125.7 ± 32.2	212.3 ± 71.8	2.13	1,10	0.180
Resting HR (beats/min)	53.8 ± 5.4	52.97 ± 6.2	55.9 ± 5.3	4.17	2,28	0.840
Breaking HR (beats/min)	100.2 ± 4.5	112.9 ± 3.2	123.9 ± 3.0	18.64	2,28	< 0.001
Eating HR (beats/min)	112.7 ± 3.5	116.7 ± 3.3	119.2 ± 3.0	2.68	2,28	0.080
Handling HR (beats/min)	110.3 ± 3.50	116.3 ± 3.2	120.4 ± 2.8	5.88	2,28	< 0.008

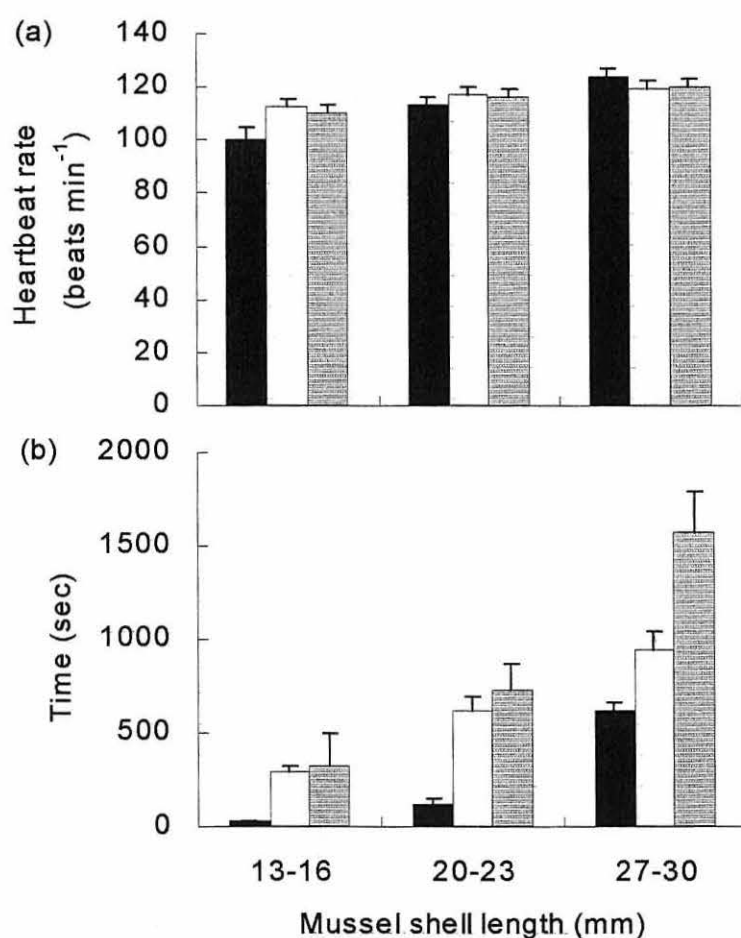


Figure 3. (a) Mean values and standard error of the heartbeat rate recorded during prey-handling behaviour (black bars = breaking, white bars = eating, grey bars = total handling, see text) by 17 crabs feeding on mussels of three size classes. (b) Mean values and standard error of duration of the same phases as in chart (a).

Table 2. Parameters of regressions of heartbeat rate on time for crabs during recovery from handling mussels of small (13-16 mm in shell length), medium (20-23 mm) and large (27-30 mm) size, respectively. Transformation refers to the type of time variable giving best fit: type 1 = Log₁₀ transformed, type 2 = untransformed. Predicted recovery is the time taken for heartbeat rate to decline to undisturbed levels, estimated using the regressions. Data are omitted when regressions were not statistically significant.

Crab number	Mussel size	Transformation	Regression constant	Regression coefficient	R ²	P	Predicted recovery (min)
1	Small	1	95.7	-12.1	0.55	<0.01	143
	Medium	2	71.5	-0.21	0.28	<0.05	171
2	Small	1	104.2	-14.8	0.60	<0.01	127
	Medium	1	114.5	-13.9	0.58	<0.01	379
	Large	2	106.0	-0.30	0.57	<0.01	243
3	Small	1	112.7	-22.1	0.94	<0.01	43
	Medium	1	100.6	-17.6	0.57	<0.01	54
	Large	1	69.1	-6.6	0.38	<0.05	42
4	Medium	1	105.5	-14.0	0.57	<0.01	410
	Large	1	60.8	-8.9	0.59	<0.01	352
5	Small	2	81.5	-0.58	0.63	<0.01	64
	Large	1	107.1	-11.2	0.60	<0.01	279
6	Small	2	67.3	-0.26	0.54	<0.01	44
	Small	1	102.3	-12.7	0.45	<0.01	80
7	Medium	1	70.6	-8.6	0.85	<0.01	17
	Large	1	85.9	-7.9	0.44	<0.01	150
	Small	1	90.2	-10.4	0.41	<0.01	146
8	Medium	1	82.3	-9.7	0.77	<0.01	91
	Small	1	117.2	-14.7	0.80	<0.01	143
9	Medium	1	77.9	-9.8	0.42	<0.01	32
	Large	1	75.4	-4.6	0.22	<0.05	93
	Medium	2	142.0	-0.68	0.47	<0.01	90
10	Large	2	132.0	-0.56	0.52	<0.01	939
	Small	1	96.7	-17.9	0.98	<0.01	23
11	Medium	1	96.8	-14.7	0.90	<0.01	45
	Large	1	121.3	-20.5	0.89	<0.01	50
	Small	1	116.9	-8.5	0.74	<0.01	184
12	Medium	1	142.0	-15.6	0.71	<0.01	85
	Large	2	122.0	-0.36	0.85	<0.01	138
	Small	2	129.0	-0.92	0.75	<0.01	45
13	Medium	2	113.0	-0.29	0.55	<0.01	88
	Large	2	114.0	-0.25	0.68	<0.01	107
	Small	2	125.0	-0.40	0.40	<0.05	76
14	Medium	2	131.0	-0.29	0.70	<0.01	117
	Small	1	104.5	-16.0	0.46	<0.05	12
	Medium	2	118.0	-0.88	0.70	<0.01	61
15	Large	1	131.9	-17.1	0.69	<0.01	55
	Small	1	100.3	-10.9	0.58	<0.01	33
	Medium	1	137.5	-15.7	0.88	<0.01	120
16	Large	2	122.0	-1.21	0.83	<0.01	50

Breaking time, eating time and therefore handling time increased significantly with prey size (Fig. 3b, Table 1). The ratio of energy content to handling time increased significantly with prey size, although the difference between medium and large prey was small (Table 1). Energetic costs of prey handling, recovery and handling plus recovery increased significantly with prey size (Table 3). The ratio of energy gain to energy cost tended to increase with prey size both for handling cost alone and for handling plus recovery costs (Fig. 4), although neither trend was statistically significant (Table 3). Gross energy gain per unit handling time increased significantly with prey size, whereas net energy gain per unit handling time showed a weaker, increasing trend with prey size that was not statistically significant (Table 3).

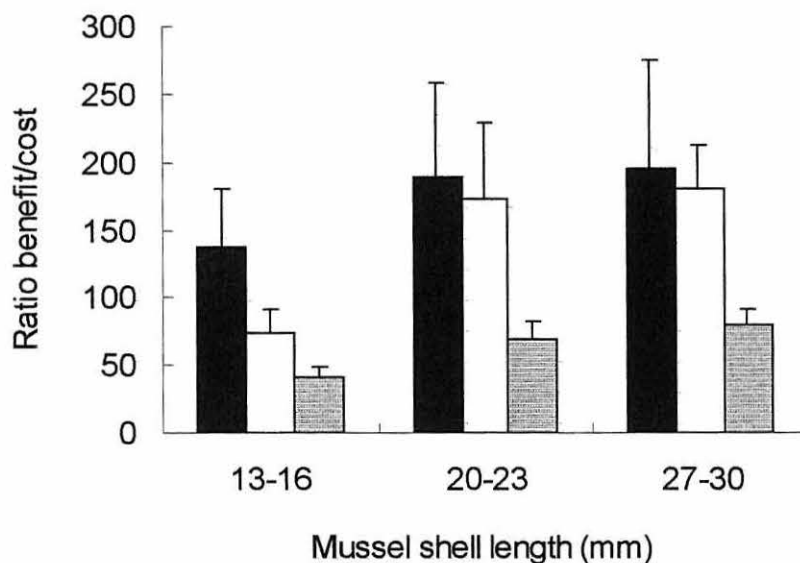


Figure 4. Mean values and standard error of ratios between energy ingested (J) and energy consumed (J) by 17 crabs during successive phases of feeding on mussels of three size classes. Black bars = handling, white bars = recovery from handling, grey bars = the sum of handling and recovery.

6.5. Discussion

Energetic costs represented only a small percentage of corresponding gains, taking mean values of 3.3%, 2.1% and 1.6% for small, medium and large mussels respectively (Fig. 4, Table 3). These costs did not include, and could not have been confounded with, post-prandial elevation of metabolic rate associated with anabolic processes, which in the shore crab begins some 4 h after prey consumption (Houlihan

Table 3. Respiratory and energetic parameters for crabs feeding on mussels. See text for explanation of parameters. Data are means with standard errors. Sample sizes are in parentheses.

	Mussel shell length (mm)			F	df	P
	13-16	20-23	27-30			
Excess O ₂ demand during handling (μmol O ₂)	5.81 ± 0.69 (17)	13.68 ± 1.52 (17)	32.18 ± 4.66 (15)	28.51	2,16	< 0.001
Excess O ₂ demand during recovery (μmol O ₂)	16.11 ± 2.59 (14)	28.82 ± 6.24 (14)	37.79 ± 8.40 (12)	5.27	2,16	< 0.020
Excess energy demand during handling (J)	2.81 ± 0.33 (17)	6.62 ± 0.73 (17)	15.57 ± 2.26 (15)	28.51	1,16	< 0.001
Excess energy demand during recovery (J)	7.79 ± 1.26 (14)	13.95 ± 3.02 (14)	18.29 ± 4.06 (12)	5.27	2,16	< 0.020
Excess energy demand during handling plus recovery (J)	10.43 ± 1.38 (14)	20.85 ± 3.27 (14)	34.62 ± 5.10 (12)	14.35	2,16	< 0.001
Ratio energy gain to cost of handling	137.9 ± 14.5 (17)	189.6 ± 24.3 (17)	195.6 ± 35.0 (15)	2.13	2,28	0.140
Ratio energy gain to cost of handling plus recovery	42.0 ± 6.8 (14)	68.8 ± 14.3 (14)	79.4 ± 13.0 (12)	2.48	2,16	0.120
Gross energy gained (J)	316.3 ± 11.4 (17)	1006 ± 32.6 (17)	2175 ± 66.2 (15)	515.9	1,20	< 0.001
Gross energy gained per unit handling time (J s ⁻¹)	1.14 ± 0.11 (17)	1.71 ± 0.20 (17)	1.77 ± 0.23 (15)	4.17	2,28	< 0.030
Net energy gained per unit handling time (J s ⁻¹)	1.20 ± 0.11 (14)	1.57 ± 0.22 (14)	1.59 ± 0.252)	0.83	2,16	0.450

et al. 1990). Similarly low prey-handling costs have been reported for juvenile blackfish, *Orthodon microlepidotus*, whose energy expenditure was only 2% of the energy content of the prey consumed when suspension feeding for *Artemia nauplii* and 1% when particulate feeding on *Artemia* adults (Sanderson & Cech 1992). Relative handling costs two orders of magnitude smaller than those above have been measured for juvenile rattle snakes, *Crotalus durissus*, feeding on mice (Cruz-Neto et al. 1999). It appears, therefore, that energetic costs of handling prey are unlikely to influence diet selection, except perhaps for predators using expensive behaviours such as prolonged pursuit.

In the case of shore crabs feeding on mussels, this inference is corroborated by a weakening of the relationship between “profitability” and prey size caused by including the energetic cost of handling prey (Table 2). Time, therefore, is the more appropriate currency for costing prey-handling behaviour of crabs. Indeed, handling time is an important basis upon which opportunistically foraging crabs select their prey. The mechanism of selection involves graded responsiveness to stimulus strength, moderated by flexible persistence time that is itself adjustable to hunger level and perhaps also to perceived risk from other predators, competitors or even of claw damage (Hughes & Seed 1995; Juanes 1992).

In conclusion, the energy maximization premise is adequately cast in terms of gross rate of energy intake. Whether behaviour is driven by maximizing the gain during available foraging time or by minimizing the time taken to acquire sufficient gain, the denominator, time, assumes critical importance both because of the principle of lost opportunity, according to which commitment to one behavioural act denies opportunity to perform others, such as searching for more profitable prey or for mates (Stephens & Krebs 1986), and because of exposure to risk of mortality (Sih 1993). Similar conclusions may extend to the cost-benefit analysis of other behavioural systems, including aggression. For example, the metabolic expenditure of crabs fighting over resources is of the same order of magnitude as the expenditure on handling molluscan prey (Rovero et al. 1999c), placing relatively light demand on the energy budget (Sneddon et al. 1999).

Chapter 7. General discussion

As in other disciplines, the fruitful study of animal behaviour depends on two important experimental characteristics: (1) the design of experiments capable of posing to animals the right “question” and (2) the use of techniques capable of recording the corresponding “answer”. This reflects the general rule that scientific progress relies as much on new ideas as on new devices for measuring, viewing or recording natural phenomena (Krebs & Davies 1984).

For each study presented in this thesis, a successful combination of the above two characteristics allowed the collection of unique data. Above all, the main suggestion generated by results is that the study of mechanisms and physiological states represents a pivotal step towards the successful quantitative investigation of adaptiveness of behavioural traits. Indeed, in the present study, the mechanistic approach made the all difference, for without it the interesting answers that came up could not have been recorded.

Thus, the direct recording of the drilling behaviour of dogwhelks (chapter 2) showed that this sub-component of prey-handling represents an almost fixed constraint on foraging, i.e. does not undergo a learning-mediated shortening of handling time, which, in contrast, occurs in other species that handle prey by less complex processes (e.g. Cunningham & Hughes 1984; Micheli 1995; Serra et al. 1997). However, dogwhelks are able to avoid the long process of shell-boring by shifting from drilling to penetration through the gaping valves, so greatly improving efficiency and halving the time of exposure to potential risks. That this alternative attack method is accomplished without application of radular rasping could not be detected without using transducers. While, for the first time, allowing quantitative analysis of the pattern of radular activity by dogwhelks (chapter 3), the externally mounted transducer ultimately represented a tool for investigating the relationship between a behavioural mechanism (radular scraping) and the related efficiency (handling time), which in turn affects fitness.

In chapter 4, monitoring heartbeat rate of mussels under exposure to effluent from dogwhelks surprisingly revealed that predation risk induces alteration of the

energetic budget irrespective of any apparent behavioural variation. Such “emotional”, risk-related alertness, not previously reported in any species, probably is adaptive in preparing the mussel for behavioural responses to potential attacks, but this could not have been detected by behavioural analysis. This paper represents a small, but critical step forward in the study of how animals balance optimization and risk avoidance.

In a similar context, when different fighting strategies of shore crabs were observed in parallel with heartbeat monitoring (chapter 5), variation in energetic costs were found to be partially due to post-fighting alertness. This state of alertness is a purely physiological response, independent of the intensity of the previous behavioural performance, and perhaps is functionally linked to perceived risk of injury and/or of losing resource ownership. By coupling cost estimation to the classic behavioural analysis, therefore, new information on the adaptiveness of fighting behaviour was collected. Among these is the evidence that the energy cost itself is not a major constraint, since time spent fighting, risk of injury and risk of losing ownership are other important factors, each presumably generating specific, mutually interacting fitness consequences. In other words, the estimation of energetic costs of behavioural decisions, while representing a new source of information, is also important for re-examining previously studied determinants of the decision-making process and for investigating their interactions.

The consistency of the above conclusion is demonstrated particularly well by the study reported in chapter 6. Monitoring heartbeat rate of crabs allowed an almost direct estimation to be made of the energetic costs of prey-handling and thus pure cost/benefit ratios could be computed. Experiments replicated those performed by Elner & Hughes (1978), in which the only currency for cost estimation was handling time. However, since costs were found to represent an almost negligible proportion of gains, it was concluded that the more pressing fitness determinant of prey-handling decisions is time.

There are strong grounds, evident throughout this study, for concluding that an integrated approach to behavioural ecology can reveal more than could be gathered from independent behavioural and physiological work. This has been previously indicated by other studies, aimed, for example, at measuring the energetic expenditure associated with various activities, as for chapter 4-6 of the present work. A variety of

methods has been implemented for that purpose, such as respirometry (e.g. Taigen & Wells 1985; Prestwich et al. 1989; Pough & Taigen 1990), whole body calorimetry (e.g. Gnaiger 1980; Shick et al. 1988; Hill et al. 1991), heart rate telemetry (Priede & Young 1977; Armøstrong et al. 1989), measure of beating rates of scaphognatites in crabs (Smith & Taylor 1993). However, because of the invasiveness or destructiveness of such methods (see also introduction of chapter 5), the analysis of behavioural components could be fairly biased, so limiting, in comparison to the present study, the power of combining physiological and behavioural approaches.

A second important conclusion is that collecting experimental data by using an integrated approach is of challenging importance for optimality modelling, as clearly shown, for example, by the study on fighting of crabs. Here the conclusion that, because of the contribution of post-fighting processes, metabolic cost was not highest in evenly size-matched fights contradicts some predictions from game theory models (e.g. Parker & Rubenstein 1981; Enquist & Leimar 1983). This contradiction is due to the fact that most of these models are purely behavioural, cost-estimation being based on variable such as time spent fighting or frequency of behavioural acts. The present study, however, suggests that non-behavioural components play an important role in cost generation, as also shown by the study of mussel responses to risk of predation (on the importance of internal state see also Houston 1993). Both experimental and theoretical investigation of the relationship between intensity of risk and related alertness costs would be required to formulate a general and coherent framework. On the other hand, chapter 6 shows that not every “non-behavioural” cost entails pressing adaptive consequences, since recovery from prey-handling ultimately did not cause significant variations on the cost-benefit balance. By stressing the importance of time as a criterion upon which animals adaptively select prey, that study represents an important vindication of classical optimal foraging models (Charnov 1976; Stephen & Krebs 1986). That optimality models, concerned with outcomes of behavioural decisions, can be successfully used as a way of investigating underlying processes has been early advocated (Krebs & Davies 1991). Moreover, stochastic dynamic programming has been developed as a theoretical framework for bringing greater realism, through inclusion of components of internal state, into modelling of decision-making processes (see Mangel & Clark 1988; Houston et al. 1988). However, greater realism entails greater complexity, this limiting the capability of formulating clear

predictions and planning coherent experiments, at least until more and more detailed physiological knowledge is accumulated (Krebs & Kacelnik 1991). The present study confirms the validity of this viewpoint and claims the need for wider inclusion, in future investigation, of underlying physiological parameters.

Referring to the opening statement of chapter 1, it seems evident, in conclusion, that in behavioural ecology “a return to mechanism does not imply turning the clock back”. Rather, this approach can help to turn the clock forward.

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Effect of experience on predatory behaviour of dogwhelks

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We used an acoustic transducer to monitor the radular activity of dogwhelks, *Nucella lapillus*, drilling mussels, *Mytilus edulis*, in the laboratory and we examined the effect of dietary experience on prey-handling behaviour. For the first time, phases of inspection, penetration and ingestion could be distinguished directly, and consequently the prey-handling process analysed in detail. Dogwhelks with different field-based experience of mussels showed different handling behaviour. Those collected from a mussel-dominated shore more readily adopted the faster method of penetrating between the slightly gaping valves, instead of the slower method of drilling through the shell. Those collected from a barnacle-dominated shore took significantly longer to attack the mussel and then were unable to switch from drilling to penetrating through the gape between valves. Experience of specific prey in the field, by reducing handling time, could promote fitness by reducing exposure to environmental hazards. Laboratory attempts to train dogwhelks from the barnacle-dominated shore to use the gape penetration method failed, suggesting that functional constraints, such as injection of a relaxant when penetrating through the gape and/or genetically controlled behavioural traits, could limit the ability to learn handling skills.

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Food acquisition and risk avoidance are critical, but often mutually conflicting, determinants of fitness (Stephens & Krebs 1986; Sih 1993). Increasing experimental evidence supports the theoretical prediction that animals are behaviourally adapted to balance food acquisition and risk avoidance (e.g. Milinski & Heller 1978; Lima et al. 1985; Hughes & Burrows 1991). Such constrained optimization of energy intake is accomplished in different ways, for example by reducing foraging activity (Lucas 1990; Burrows & Hughes 1991), by appropriate choice of prey, or by reducing prey-handling time (Elner & Hughes 1978; Palmer 1983). Specifically, intertidal species that feed on hard-shelled prey, for example muricid gastropods and predatory crabs, face persistent handling constraints which, together with the need to avoid mortality, influence foraging behaviour (Lawton & Hughes 1985; Brown & Richardson 1987). These species are generally able to improve handling skills through experience of prey, so shortening handling time (Cunningham & Hughes 1984; Hughes & Dunkin 1984;

Micheli 1995; Serra et al. 1997). Despite the theoretical importance of learned improvement in prey-handling skills (e.g. Hughes 1979), the underlying mechanisms remain poorly understood, probably because of difficulties in quantifying the complex handling processes involved (Fairweather & Underwood 1983; Godin 1990; Hughes & Seed 1995). Increasing this understanding would represent an important step towards a progressively more integrated analysis of the relationship between foraging behaviour and fitness (Houston et al. 1988).

The North Atlantic muricid gastropod *Nucella lapillus* is an ideal subject for investigating the influence of experience on handling behaviour. Dogwhelks forage almost exclusively on barnacles and mussels by drilling the shell, using combined radular scraping and chemical dissolution (Crothers 1985). Handling times of these sessile prey can span several tidal cycles (Hughes & Drewett 1985), thus influencing exposure to environmental hazards such as dislodgement by waves (Etter 1996) and predation (Palmer 1990; Vadas et al. 1994). Experience of prey can greatly reduce handling times for barnacles (Dunkin & Hughes 1984) and mussels (Hughes & Dunkin 1984). Precisely how experience affects handling time in this way remains unknown, since drilling has never been directly recorded and is normally indistinguishable from

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ingestion (Bayne & Scullard 1978). Attempts to monitor directly the drilling activity of a predatory gastropod have been made only on the muricid *Urosalpinx cinerea*, by using microhydrophones to record the noise made by the radula scraping the prey's shell (Carriker & Martin 1965; Carriker & Van Zandt 1972). Recently, this technique has been enhanced for accurately monitoring the grazing activity of herbivorous chitons and limpets (Parpagnoli & Chelazzi 1995) and we used it in the present study to monitor continuously the radular activity of dogwhelks feeding on mussels. In this way, behavioural components of the prey-handling process could be quantified and the influence of experience on these processes examined. The study illustrates how a mechanistic approach to the analysis of behavioural processes, not amenable to field monitoring, can aid investigation of the adaptive interaction between constraints (handling of prey) and state variables (level of experience).

METHODS

General Methods

We collected dogwhelks before each experimental session from two rocky shores on Anglesey, North Wales, U.K. The first shore, Red Wharf Bay, was densely populated by barnacles and mussels, whereas the second, Menai Bridge, was populated only by barnacles. We therefore assumed that dogwhelks from the first population were experienced at handling both barnacles and mussels, while dogwhelks from the second population were inexperienced with mussels. Only adult dogwhelks were collected, recognizable by their apertural teeth and eroded apertural margin (Crothers 1985).

Mussels 25–30 mm in shell length were collected every 2 weeks from a sandy shore near Bangor, North Wales, U.K. We considered this size large enough to apply sensors (below) and it is known to be selected by adult dogwhelks both in the laboratory (Hughes & Dunkin 1984) and in the field (Hughes & Drewett 1985). Barnacle-covered rocks, about 10 cm in diameter, were used for maintaining the control batches of dogwhelks and were collected from the same site as the mussels. Prey were fed continually with *Rhinomonas reticulata*. Except for the mussels offered to the dogwhelks as prey, all individuals used in the experiments were eventually released at the collection sites.

We conducted experiments from January to June 1998 at $17 \pm 2^\circ\text{C}$ under continual illumination. The experimental aquaria contained recirculating, aerated, filtered sea water that was changed every 2 weeks.

Video and Drilling Recording

A camera placed above the experimental aquarium was connected to a time-lapse video recorder, capturing frames at 3-s intervals. A piezoelectric transducer was fixed with cyanoacrylate adhesive to one of the valves of the mussel, close to the dorsal hinge. The opposite valve was glued to a PVC base, forcing dogwhelks to drill the

upper valve bearing the sensor, so maximizing the resolution of the signal. The stable, horizontal position of the mussel facilitated video monitoring of behavioural patterns. We checked the correct digital encoding of radular activity for each predatory event by comparing digital with audio signals that were monitored by tape recording and/or by listening through headphones. When audio signals, forming a sequence of pulses as the radular teeth scraped over the mussel shell, corresponded to an equal number of digitally encoded events, the recording was considered valid. We discarded recordings of any predatory event accompanied by extraneous noise or by signals of low resolution. Data were stored automatically on a computer and files exported every 48 h for processing (Parpagnoli & Chelazzi 1995), yielding the number of radular rasps of each dogwhelk, both in a given interval of time and throughout the predatory sequence.

Experimental Protocol

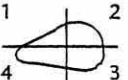
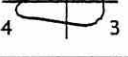
The experimental aquarium was divided by plastic mesh into six compartments each of 10×10 cm, representing three experimental and three control treatments. In each compartment we placed a dogwhelk of known shell height and a mussel of known shell length, bearing a transducer. Predator-prey pairs were replaced once a dogwhelk had abandoned the mussel, having completed its attack. We used a $40\times$ dissection microscope to see in which quadrant of the shell the borehole had been made (see Hughes & Dunkin 1984 and diagram in Table 1) and to see if partial holes or other drilling marks were present. Shell thickness at the borehole was measured with a vernier micrometer. We compared observed frequencies of drilling site location with those expected from non-selective drilling, that is, an equal number of bored holes per quadrant, by using chi-square tests (Siegel & Castellan 1989). We also used chi-square tests to compare observed distributions of drilling site location for mussel-experienced and mussel-inexperienced dogwhelks.

We classified attacks according to whether the dogwhelks drilled a hole through the shell of the mussel (bored hole method) or penetrated the slightly gaping valves (gape insertion method). Inspection of the empty mussel valves, supplemented by video analysis (see below), allowed us to assign attacks unambiguously to one of the two categories. Cases of hole drilling at the edge of the valve were assigned to the gape insertion method. Differences in the relative occurrence of attack methods between experimental and control batches were compared by chi-square tests.

Experiment 1: effect of field-based experience

To test whether a long-term diet history affects handling times, we compared the foraging behaviour of dogwhelks from Red Wharf Bay (mussel experienced) and Menai Bridge (mussel inexperienced). We first deprived the dogwhelks of food for 4 weeks, to standardize hunger levels. The experiment then ran for 10 weeks, until at least 20 attacks per treatment had been monitored.

Table 1. Number of predation events per attack method and number of bored hole attacks per drilling site on the shell of the mussel (see bottom left diagram), for mussel-experienced (E) and mussel-inexperienced (I) dogwhelks

		Experiment 1		Experiment 2		Experiment 3	
		E	I	E	I	E	I
Attack method	Hole	26	30	19	25	79	119
	Gape	17	3	5	2	50	13
Drilling site	1	12	9	9	9	39	49
1  2	2	10	10	8	9	30	42
	3	1	5	2	3	6	13
4  3	4	4	6	1	3	4	15

Hole: penetration through a drilled hole; gape: penetration between the gaping valves. Experiment 1 compared dogwhelks that had or did not have experience with mussels in the field; in experiment 2, the dogwhelks did not have field experience with mussels but half were given experience in the laboratory; experiment 3 was similar to experiment 1 but the mussels were given in clusters, instead of singly.

Experiment 2: effect of laboratory-based experience

Dogwhelks from the Menai Bridge population, inexperienced with mussels, were divided into two batches and maintained for 9 weeks in aquaria with mussels (experimental batch) or barnacles (control batch), respectively. Dogwhelks maintained with mussels had eaten an average of nine mussels by the end of dietary training and thus were assumed to have become experienced in handling mussels. After dietary training, we deprived the dogwhelks of food for 2 weeks to standardize hunger levels. This relatively short period of food deprivation was used in order to minimize attenuation of learned prey-handling skills. The experiment then ran for 8 weeks, until at least 20 attacks per treatment had been monitored.

Experiment 3: attack methods on aggregated mussels

In this experiment we tested whether the occurrence of attack methods in experiments 1 and 2 could have been biased by the isolation and unnatural orientation of mussels cemented to the substratum. Two batches of 50 dogwhelks from Menai Bridge and Red Wharf Bay populations (mussel inexperienced and mussel experienced, respectively) were deprived of food for 2 weeks, and then maintained in aquaria with 25–30-mm mussels that had been allowed to aggregate into clusters of 20–30 individuals, until a minimum of 100 attacks per batch had been recorded. We inspected mussels regularly and classified empty shells for attack method and drilling site selection. The experiment lasted 9 weeks.

Assessment of Parameters of Foraging Behaviour

Prey-handling behaviour of *N. lapillus* consists sequentially of inspection, drilling and ingestion (Hughes & Dunkin 1984). Video and drilling recording produced complementary information that, once integrated, enabled us to quantify the three prey-handling phases (Fig. 1).

Video analysis

We analysed each attack from the moment the dogwhelk was placed with the mussel to the moment it

abandoned the prey. Prey-handling phases were measured in minutes and then converted to hours, to two decimal places. The period elapsing until the dogwhelk made contact with the mussel was defined as the time before attack; the phase of exploratory movements of the dogwhelk on the shell of the mussel before it adopted a consistent drilling position was considered as inspection time. If the prey was attacked, a phase of indistinguishable drilling and ingestion was quantified. Total handling time was therefore given by inspection time + drilling and ingestion time.

Any other movements of the dogwhelk or mussel were recorded. In particular, we used the cessation of valve gaping or foot extension by the mussel, together with drilling analysis data (below), to estimate the beginning of ingestion.

Drilling analysis

Within a predatory sequence, the period in which radular activity occurred was defined as drilling time. It was also expressed as a fraction of total handling time (drilling time/handling time). Any interval from the beginning of inspection to the beginning of drilling was defined as predrilling time, whereas the phase that followed drilling time was defined as ingestion time.

We quantified drilling activity by scoring the total number of radular rasps (Fig. 1). An index of the intensity of drilling was expressed as the number of radular rasps/h, given by the ratio number of radular rasps/drilling time.

We used the two-tailed Mann-Whitney test for unpaired data (Siegel & Castellan 1989) to compare foraging parameters of experimental and control dogwhelks and differences between attack methods within batches. Analysis of covariance (Sokal & Rohlf 1995) was used to examine potential effects of dogwhelk size, mussel size and starvation period on handling time. Pearson correlation analysis was used to examine relationships between the above covariates and behavioural measures (Sokal & Rohlf 1995).

Unless specified, values are given as means \pm SE.

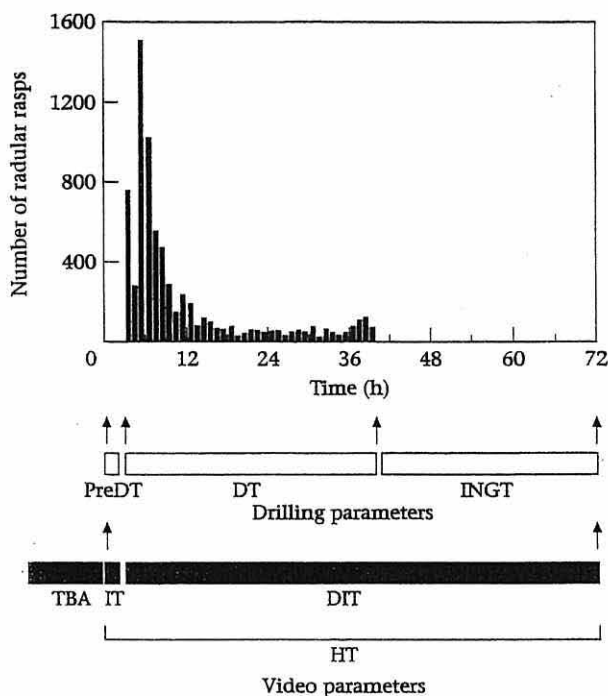


Figure 1. Diagram of criteria used for assessing components of behaviour of dogwhelks feeding on mussels, based on integrated drilling and video data. Drilling time (DT) is the period characterized by radular activity, estimated by the number of radular rasps. Predrilling time (PreDT) and ingestion time (INGT) are the periods before and after drilling time, respectively. From the video parameters, total handling time (HT) was partitioned into inspection (IT) and drilling and ingestion time (DIT), which themselves cannot be separated. Time before attack (TBA) is the period elapsing from placement of the dogwhelk in the foraging compartment to the start of the attack.

RESULTS

Attack Method

In experiment 1, the gape insertion method was used significantly more frequently by dogwhelks from Red Wharf Bay, which had gained experience of mussels in the field, than those from Menai Bridge, which had not (Table 1; chi-square test: $\chi^2_1=8.92$; $P<0.005$). For Red Wharf Bay dogwhelks, handling time was 53% shorter for the gape insertion than for the bored hole method (Table 2, Fig. 2; Mann-Whitney test: $U=788.0$, $N_1=17$, $N_2=26$, $P<0.0001$). For Menai Bridge dogwhelks, handling time was 48% shorter for the gape insertion method, but this difference was not tested statistically because of the small number of gape insertion attacks recorded.

In experiment 2, the gape insertion method was used too infrequently for statistical analysis (Table 1). With naturally aggregated mussels (experiment 3), gape insertion attacks were used significantly more frequently by experienced than by inexperienced dogwhelks (Table 1; chi-square test: $\chi^2_1=29.78$, $P<0.001$). Relative frequencies of gape insertion and bored hole methods were not significantly different between experiment 1,

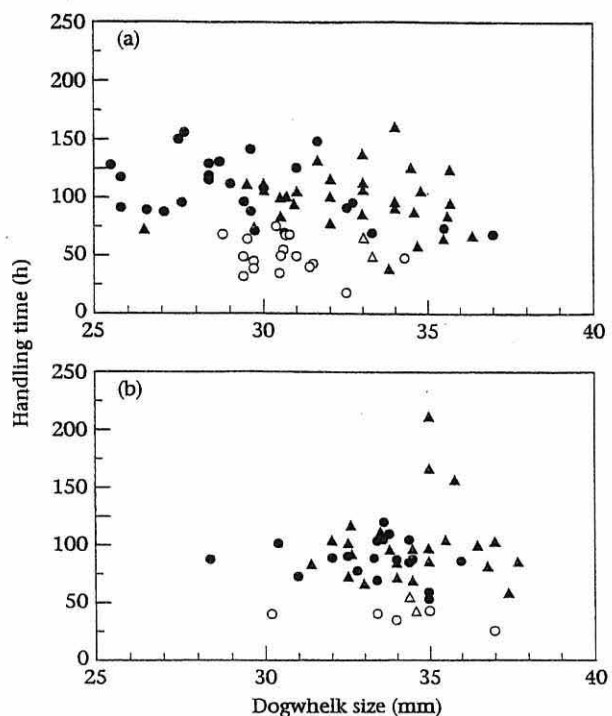


Figure 2. Handling time (h) plotted against dogwhelk size (mm in shell length) for (a) experiment 1 and (b) experiment 2. Values are categorized by experience of the dogwhelk, i.e. previously having attacked mussels (mussel experienced; ●, ○) or having attacked only barnacles (mussel inexperienced; ▲, △), and by attack method used, i.e. drilling a hole through the shell (hole; ●, ▲) or penetrating between the slightly gaping valves (gape; ○, △). See Table 1 for explanation of experiments.

when mussels were isolated, and experiment 3, when they were aggregated (Table 1; chi-square test: $\chi^2_1=0.008$, $P=0.92$).

No significant correlation was found between starvation period and handling time. As expected from the small range of prey sizes used, handling time was not significantly correlated with mussel size. Handling time was significantly correlated with dogwhelk size only for mussel-experienced dogwhelks using the bored hole method in experiment 1 (Pearson correlation: $r=-0.42$, $N=26$, $P<0.03$). Also in experiment 1, mussel-experienced dogwhelks using the gape insertion method were significantly larger than those using the bored hole method (Fig. 2, Mann-Whitney test: $U=455.0$, $N_1=17$, $N_2=26$, $P<0.05$).

In experiment 2, as in experiment 1, handling time for the gape insertion method (52 ± 2.96 h) was significantly shorter than for the bored hole method (89.08 ± 4.03 h; Table 2; Mann-Whitney test: $U=285.0$, $N_1=5$, $N_2=19$, $P<0.001$). This comparison is based only on dogwhelks experienced with mussels, since too few mussel-inexperienced dogwhelks used the gape insertion method.

Although, in experiment 1, dogwhelks from Menai Bridge were larger than those from Red Wharf Bay, this

Table 2. Video parameters per attack method for mussel-experienced and mussel-inexperienced dogwhelks

Batch	Method	N	Time before attack (h)	Inspection time (h)	Handling time (h)
Experiment 1	Experienced	Hole	4.04±1.03	2.35±0.43	106.8 ±5.25
		Gape		1.40±0.73	49.7 ±3.68
		Total			
	Inexperienced	Hole	13.35±2.75	1.35±0.24	100.51±4.18
		Gape		1.33±1.22	51.53±7.69
		Total			
Experiment 2	Experienced	Hole	20.09±4.26	1.03±0.33	89.08±4.03
		Gape		0.08±0.07	36.52±2.96
		Total			
	Inexperienced	Hole	21.47±7.19	0.54±0.12	99.67±6.85
		Gape		0	48.62±6.29
		Total			

Values are $\bar{X} \pm \text{SE}$. Hole: Penetration through a drilled hole; gape: penetration between the gaping valves. See Table 1 for explanation of experiments.

difference did not significantly confound comparisons of handling time (experiment 1; ANCOVA: $F_{51,1}=2.40$, $P=0.128$). Experience had no significant effect on handling time for the bored hole method (Fig. 2; Mann-Whitney test: experiment 1: $U=791.0$, $N_1=26$, $N_2=30$, $P=0.41$; experiment 2: $U=578.0$, $N_1=19$, $N_2=25$, $P=0.72$).

Drilling Site Selection

Drilling sites on the shell of the mussel were significantly nonrandomly distributed for dogwhelks experienced with mussels in experiment 1 (chi-square test: $\chi^2_3=15.83$, $P<0.01$) and for both mussel-experienced and mussel-inexperienced batches in experiment 3 (chi-square test: experienced: $\chi^2_3=41.05$, $P<0.001$; inexperienced: $\chi^2_3=27.54$, $P<0.001$). Quadrants 1 and 2, on the hinge side of the valve, were selected more frequently for drilling than quadrants 3 and 4, but there was no significant difference in usage of these quadrants by mussel-experienced and mussel-inexperienced dogwhelks (Table 1).

Temporal Parameters and Radular Activity

In experiment 1, dogwhelks inexperienced with mussels took significantly longer than mussel-experienced individuals to attack each mussel (Table 2; Mann-Whitney test: $U=1026$, $N_1=33$, $N_2=43$, $P<0.005$), whereas in experiment 2 time before attack was not significantly different between batches. Once contacted, the mussel was initially inspected around the gape between valves. In experiment 1, for mussel-experienced dogwhelks, inspection time was significantly shorter for the gape insertion than for the bored hole method (Mann-Whitney test: $U=637.0$, $N_1=17$, $N_2=26$, $P<0.05$). Gape insertion attacks by mussel-inexperienced dogwhelks were too few for statistical analysis, but again tended to involve shorter inspection time than bored hole attacks (Table 2). For the bored hole method, inspec-

tion time was not significantly different between mussel-experienced and mussel-inexperienced dogwhelks.

Direct observation together with headphone monitoring of radular activity suggested that radular activity began within 1–5 min of inspection (Fig. 1). Consequently, for all batches, there was no significant difference between inspection time and predrilling time.

Drilling activity patterns differed between attack methods (Table 3). In several gape insertion attacks no radular activity was recorded, but since low resolution of the signal could not be excluded as the cause, these cases were not used in computing the values reported in Table 3. The remaining data showed that gape insertion attacks required less drilling activity, measured both as drilling time and as number of radular rasps. For dogwhelks experienced with mussels in experiment 1, drilling time for the gape insertion method was significantly shorter than for the bored hole method (Mann-Whitney test: $U=260.0$, $N_1=N_2=13$, $P<0.0001$). Similarly, the ratio drilling time/handling time was significantly less for the gape insertion method than for the bored hole method ($U=99$, $N_1=N_2=13$, $P<0.005$). Number of radular rasps was marginally not significantly less for the gape insertion method than for the bored hole method ($U=211.0$, $N_1=N_2=13$, $P=0.07$). Consequently, the intensity of radular activity, that is, the ratio number of radular rasps/drilling time, was significantly less for the bored hole method than for the gape insertion method ($U=123.0$, $N_1=N_2=13$, $P<0.01$).

Drilling time for the bored hole attack method was not significantly different between mussel-experienced and mussel-inexperienced dogwhelks (experiment 1: $U=201.0$, $N_1=13$, $N_2=15$, $P=0.58$; experiment 2: $U=143.5$, $N_1=12$, $N_2=11$, $P=1$). However, in experiment 1, radular activity was marginally not significantly less for mussel-experienced than for mussel-inexperienced dogwhelks (number of radular rasps: $U=149.0$, $N_1=N_2=13$, $P=0.08$, one-tailed $P<0.05$; number of radular rasps/drilling time: $U=148.0$, $N_1=N_2=13$, $P=0.08$, one-tailed $P<0.05$). This

Table 3. Drilling parameters, for mussel-experienced and mussel-inexperienced dogwhelks

	Experiment 1				Experiment 2		
	Experienced		Inexperienced		Experienced		Inexperienced
	Hole	Gape	Hole	Gape	Hole	Gape	Hole
<i>N</i>	13	13	15	2	12	14	11
Drilling time (h)	57.99±3.56	14.06±2.77	56.12±4.51	12.15±5.04	55.80±4.08	12.62±4.08	54.38±4.29
No. radular rasps	2128±334	1753±657	4191±895	870±271	3976±1062	1606±714	3409±884
No. radular rasps/drilling time	41.74±8.93	107.28±16.15	79.76±18.47	97.84±63.01	71.26±16.47	124.80±29.03	60.93±13.56
Drilling time/handling time	0.56±0.02	0.28±0.04	0.57±0.02	0.22±0.04	0.62±0.03	0.31±0.09	0.61±0.05
Predrilling time (h)	3.09±1.62	1.44±0.64	1.65±0.37	3.20±1.80	1.37±0.36	1.72±1.06	1.05±0.24
Ingestion time (h)	42.52±0.27	31.87±3.45	39.97±4.00	37.25±6.34	31.77±2.89	24.85±3.67	38.80±10.55

Values are $\bar{X} \pm \text{SE}$. Hole: Penetration through a drilled hole; gape: penetration between the gaping valves. See Table 1 for explanation of experiments.

trend was not reflected in experiment 2 (number of radular rasps: $U=153.0$, $N_1=12$, $N_2=11$, $P=0.60$).

Mussel shell thickness at the borehole ranged from 0.5 to 0.9 mm and was not significantly different in mussels presented to experienced and inexperienced dogwhelks (Mann-Whitney test: $U=1617.5$, $N_1=45$, $N_2=55$, $P=0.50$). Drilling time was significantly correlated with shell thickness only for mussel-inexperienced dogwhelks of experiment 1 (Pearson correlation: $r=0.645$, $N=15$, $P<0.01$). Penetration rate, estimated as shell thickness/drilling time was 0.29 ± 0.009 mm/day for all experimental batches. Handling time was significantly correlated with shell thickness for dogwhelks inexperienced with mussels in experiment 1 (Pearson correlation: $r=0.582$, $N=30$, $P<0.01$) and for mussel-experienced dogwhelks in experiment 2 ($r=0.677$, $N=19$, $P<0.002$).

For dogwhelks experienced with mussels in experiment 1, ingestion time was marginally not significantly shorter for the gape insertion attack method than for the bored hole method (Table 3; Mann-Whitney test: $U=214.0$, $N_1=N_2=13$, $P=0.05$).

DISCUSSION

The technique we used for monitoring drilling activity has allowed, for the first time, the different components of prey handling by dogwhelks to be distinguished. This in turn has enabled us to examine the effect of dietary experience on the prey-handling process in greater detail.

Greatest behavioural contrast was found in experiments 1 and 3, between dogwhelks with different field-based experience of mussels: those from Red Wharf Bay, where mussels and barnacles were abundant, more readily adopted the faster, gape insertion method than those from Menai Bridge, where only barnacles were present. We believe that dietary experience is the principal factor explaining the greater use of the shorter handling method by dogwhelks from the mussel-dominated shore. Even though risk of predation is higher at Menai Bridge, a sheltered shore with high crab density (personal observation), dogwhelks from that shore did not use the shorter handling method. Although Red Wharf Bay is a moderately exposed shore (with relatively lower crab density), wave impact is unlikely to pose a major risk to foraging dogwhelks. We therefore discount risk of predation or dislodgement by waves as potentially confounding factors in our interpretation of population differences in prey-handling behaviour. Empty mussel valves, whose posterior margins show signs of drilling that characterize the gape insertion method, are frequently found among smaller size classes on mussel-dominated shores (Hughes & Burrows 1993; personal observation). Since, in the present experiments, mussels were almost totally consumed, switching from the bored hole method to the faster gape insertion method doubled the feeding rate. While potentially doubling the energy gain per foraging bout, the reduction in handling time could also halve the exposure time to environmental hazards, so promoting fitness under many commonly occurring field conditions (Burrows & Hughes 1990).

Although in experiments 1 and 3 the gape insertion method was used more frequently by dogwhelks already experienced with mussels in the field, it was never used exclusively. When mussel-experienced dogwhelks used the alternative bored hole method they took just as long as mussel-inexperienced individuals to drill the prey. This matches Hughes & Dunkin's (1984) finding that whereas dogwhelks from a barnacle-dominated shore learned to increase efficiency in handling smaller mussels by drilling thinner parts of the shell, they did not do so for larger mussels spanning the size range used in the present experiments. Nevertheless, dogwhelks experienced with mussels performed fewer radular rasps than inexperienced dogwhelks. This difference became significant with a one-tailed test, which is justified if it is considered that experience could only increase foraging efficiency. Drilling time was conserved perhaps through changes in the efficiency of chemical dissolution, used alternately with scraping (Carriker 1981). Whether or not this reduction in radular activity makes significant savings in energy cannot be assessed without appropriate metabolic data.

In contrast to experiments 1 and 3, laboratory-based experience of mussels had no significant effect on the frequency of attack methods used by dogwhelks in experiment 2. Many studies have addressed the effect of experience on prey-handling behaviour (review in Hughes et al. 1992; see also Hughes & Croy 1993; Serra et al. 1997) and in most of them increasing experience with a particular type of prey resulted in greater handling efficiency. Typically 5–10 attacks are required for handling skills to reach an asymptote in animals as diverse as fish, crabs and snails. Since similar numbers of prey were attacked by dogwhelks in experiment 2, it is puzzling why handling efficiency showed no significant improvement. Perhaps learning to adopt the gape insertion method requires a longer sequence of encounters with small-to-medium sized mussels.

The gape insertion method presumably relies upon effective application of toxin (Andrews 1991) to the mantle rim exposed by initial drilling. The majority of gape insertion attacks in the present study involved little drilling, and evidence of scraping was often apparent only under the dissection microscope. Drilling may therefore be limited to the first exposure of tissue, when toxin can be applied. Video analysis revealed that the valves of the mussels were gaping slightly during ingestion, presumably as a result of narcotization. It is unlikely that the accessory salivary glands hold a sufficient dose of toxin to be effective on large mussels, a conclusion corroborated by experiment 1 where gape insertion attacks were made by significantly larger dogwhelks (see also Serra et al. 1997). Encounters with prey unsuitable for the gape insertion method may reinforce continued adoption of the bored hole method, which is ultimately successful with any prey and so is more suitable as a general attack strategy.

A process analogous to the gape insertion method is used by dogwhelks experienced in attacking barnacles, where penetration becomes focused on sutures between the skeletal plates (Hughes & Dunkin 1984). Behavioural selection of the penetration site, however, probably

differs according to the morphological disparity between barnacles and mussels, rendering skills nontransferable (Osgood 1949) between these prey types. Long-term dietary history may have caused a lasting behavioural effect that could not be altered by the relatively short-term experience staged in the laboratory. Behavioural traits might also be genetically controlled (Hughes & Taylor 1997), perhaps hindering adjustment to alien prey. If so, this might be clear evidence of small-scale genetic differentiation in dogwhelk populations as a consequence of direct development and localized differences in chromosome number (Dixon et al. 1994). Laboratory rearing experiments will be required to resolve this problem.

The present study illustrates the value of adopting a mechanistic approach to the analysis of foraging behaviour, where an understanding of decision-making processes requires detailed knowledge of ways in which constraints and state variables influence subcomponents of the prey-handling process (Burrows & Hughes 1991; Houston 1993).

Acknowledgments

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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 (Chetail 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

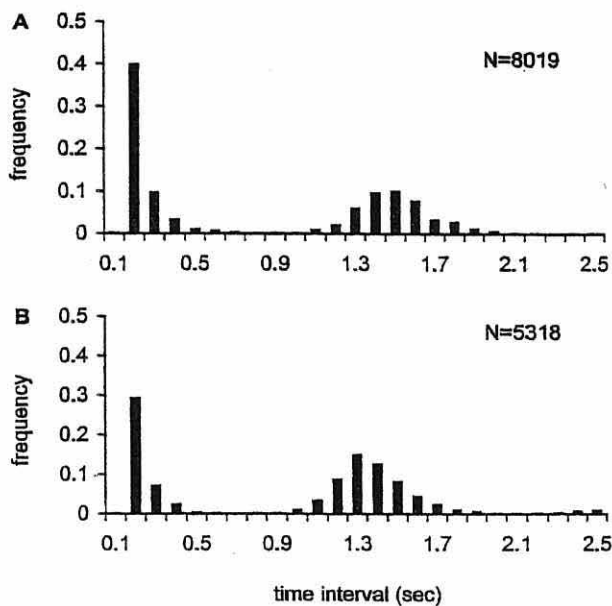


Figure 1. Frequency distribution, for a range of 2.5 s, of time intervals between successive unit events of radular rasping during two drilling processes by dogwhelks on mussel shells, (A) and (B). Each unit event probably correspond to the application of one row of radular teeth to the mussel shell. 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).

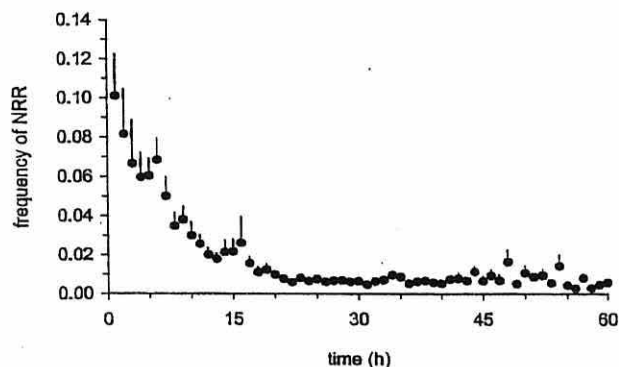


Figure 2. Frequency distribution of number of radular rasps (NRR) h^{-1} recorded for 21 dogwhelks drilling on mussel's shells. Values are plotted along a 60 h range, during which period the shell penetration process generally is accomplished. Data are means (\pm SE).

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 $17 \pm 2^\circ\text{C}$ under continual illumination. Aquaria contained recirculating, filtered and aerated seawater which was replaced with freshly collected seawater every two weeks.

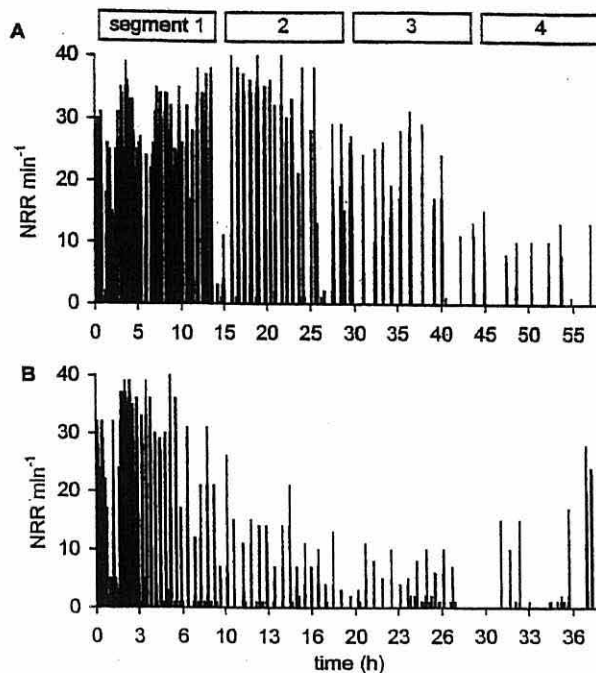


Figure 3. Time series of number of radular rasps (NRR) min^{-1} , plotted for two drilling sequences, (A) and (B), throughout the drilling process. Each sequence is divided into four segments (see text for explanation) of equal duration, as shown for the attack (A).

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.

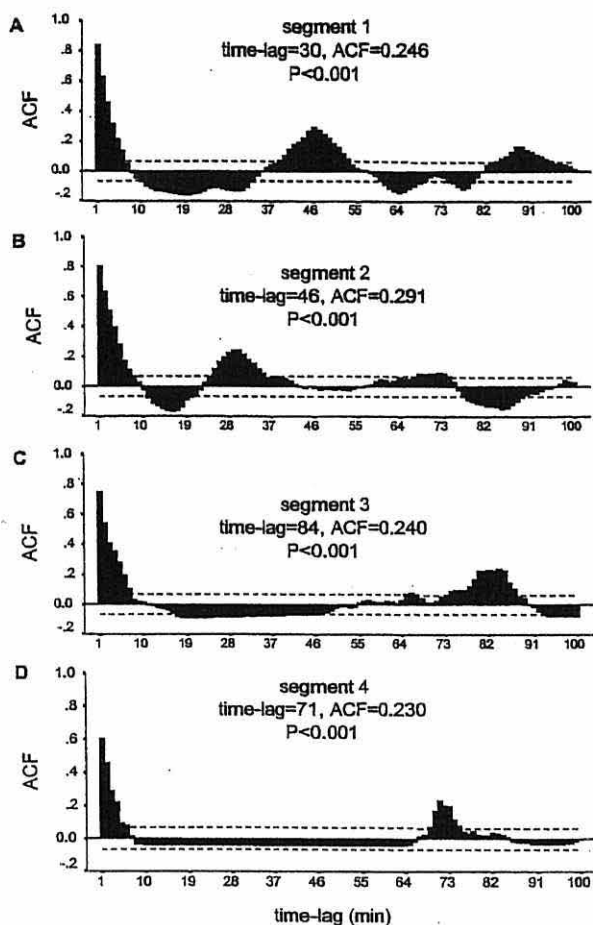


Figure 4. Values of autocorrelation coefficient (ACF) from the analysis of each temporal segment of the drilling sequence shown in Figure 3A. Horizontal dashed lines show 95% confidence limits. Time lags (min) corresponding to the higher ACF are reported in the charts, and represent the significant periodicity 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, 2, 3 and 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 $(\text{EDT} \times \text{Periodicity}) / \text{DT}$, 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.

Parameter	Temporal segment				P
	1	2	3	4	
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 (Norusis, 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 2–4 (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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Cardiac and behavioural responses of mussels to risk of predation by dogwhelks

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We used noninvasive, infrared phototransducers to record continuously the heartbeat of common mussels, *Mytilus edulis*, experiencing successive phases of interaction with the predatory gastropod *Nucella lapillus*, from initial threat to attack and consumption. Coupling physiological monitoring with behavioural analysis allowed us to investigate in detail the responses of mussels to predation threat. Compared to values of normal feeding activity, heart rate increased significantly when mussels were in the presence of effluent from dogwhelks. When attacked by dogwhelks, mussels increased their heart rate further, together with the rate of valve gaping. Considering the heart rate as a reliable estimator of respiratory function, these cardiac responses might be a mechanism to compensate for increased energy demand in order to cope with predation hazard. If so, the theoretical importance of trading off energy balance against risk of predation is supported by our results. Cardiac and behavioural responses varied throughout the attack according to the penetration method adopted by dogwhelks. When mussels were attacked through a drilled hole, heart rate tended to increase and periods of cardiac pausing appeared close to the point of death. In contrast, mussels attacked by penetration between the valves showed decreasing heart rate throughout the attack, together with relatively earlier cessation of valve gaping and appearance of cardiac pausing. These differences clearly support the hypothesis that dogwhelks penetrating between the valves are able to induce muscular paralysis of prey by injecting toxins.

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Monitoring heart rate has been used extensively to study physiological responses of invertebrates to environmental fluctuations. For bivalve molluscs and other marine taxa in particular, investigations have addressed the effects of physicochemical variables, such as temperature, salinity, exposure to hypoxia and air (see Bayne et al. 1976; DeFur & Mangum 1978; Shick et al. 1986; Marshall & McQuaid 1993). Few data are available, however, on physiological responses of subjects to perceived risk of predation (but see Metcalfe et al. 1987) or even to actual predatory attack. On the other hand, predation threat has been the object of much behavioural research, which theoretically and experimentally has shown how predation risk constrains the decision-making processes of prey (reviews in Godin 1990; Lima & Dill 1990; Sih 1993). Attempts to estimate the fitness consequences of coping with

predation threat have been made by quantifying changes in the time budget or behavioural efficiency, for example involving refuging, increased vigilance and associated reductions in foraging opportunity (e.g. Milinski & Heller 1978; Abrahams & Dill 1989; Vadas et al. 1994; Serra et al. 1997). An ideal experimental approach, however, would require behavioural responses to be monitored together with one or more estimators of the prey's physiological status, such as heart rate. This would allow the physiological demands of different responses to be directly inferred and therefore fitness consequences analysed in greater detail. New techniques for noninvasive monitoring of physiological states of selected invertebrates (see Depledge & Andersen 1990; Aagaard et al. 1991) potentially allow the above approach to be achieved, since animals performing normal activity can be monitored. We attempted this by monitoring in the laboratory both cardiac and behavioural responses of common mussels, *Mytilus edulis*, under successive phases of interaction with a predatory muricid gastropod, the dogwhelk, *Nucella lapillus*, from initial perceived risk of predation to final attack.

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The common mussel is widely distributed in the high and mid-intertidal of northern hemisphere shores (Seed 1976). The cardiac physiology of *M. edulis* has been studied for many years using invasive techniques such as electrode implantation or visual inspection through a hole drilled through the shell (review in Bayne et al. 1976; see also Shick et al. 1986). Only recently has a noninvasive technique been tested on this species (Haefner et al. 1996). Although the relationships between heartbeat and metabolic rates are still not fully understood (Booth & Mangum 1978; Famme 1981), it is generally accepted that for mussels heart rate is a reliable estimator of respiratory function (Bayne 1971; Coleman 1974; Marshall & McQuaid 1993).

The dogwhelk is one of the major predators of mussels (Seed 1976) and readily attacks prey in the laboratory (Hughes & Dunkin 1984). The prey-handling process can last several days, since it usually involves drilling the prey's shell before ingesting. Dogwhelks from mussel-dominated shores, however, can greatly shorten handling times by inserting the proboscis through the gaping valves (Rovero et al. 1999).

METHODS

General Methods

Mussels, 28–32 mm in shell length, were collected from a sandy beach near Bangor (North Wales, U.K.) and maintained for a month, before the experiments, in aquaria on a diet of *Rhinomonas reticulata*. This prolonged maintenance in controlled conditions was planned to avoid any possible endogenous variation of heart rate during the experiments, caused by tidal and/or diurnal rhythms (F. Rovero, unpublished data). The size chosen was large enough for the application of transducers (see below) and is commonly accepted by adult dogwhelks both in the laboratory (Hughes & Dunkin 1984) and in the field (Hughes & Drewett 1985).

We collected adult dogwhelks, 35–40 mm in shell height, from Red Wharf Bay, a rocky shore on Anglesey (North Wales) densely populated by mussels. Each dogwhelk was deprived of food for 2 weeks to standardize hunger level, and then monitored while attacking a mussel. In the field, dogwhelks may endure even longer periods without feeding, particularly during harsh environmental conditions (Burrows & Hughes 1991).

We conducted experiments from September to December 1998 at $17 \pm 1^\circ\text{C}$ under continual illumination. Experimental aquaria ($25 \times 40 \times 30$ cm) contained 10 litres of recirculating, aerated sea water regularly supplied with *R. reticulata* to give an almost constant food concentration of about 500 cells/ml.

Techniques

The noninvasive technique for monitoring heart rate was similar to that introduced by Depledge & Andersen (1990). The sensor consisted of a phototransistor axially coupled with an infrared light-emitting diode, fixed with

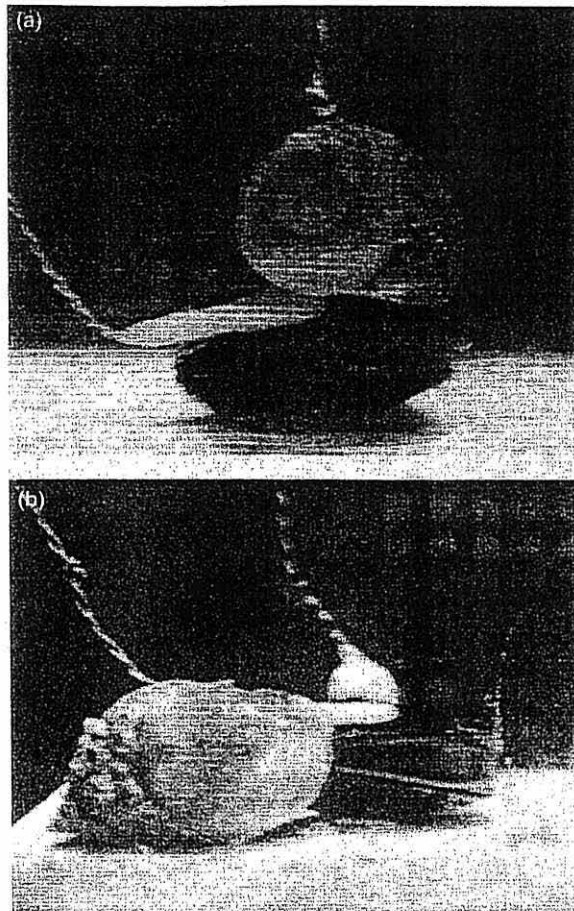


Figure 1. Photographs of mussels being attacked by dogwhelks (a) drilling a hole through the shell of the mussel and (b) penetrating between the gaping valves. Transducers are glued to the superior valve of the mussel. The elongated one (a) is a piezoelectric transducer for recording radular activity by the dogwhelk, the cubic one (b), close to the dorsal hinge, is a phototransducer for monitoring heartbeat.

cyanoacrylate glue to the shell of the mussel in the dorsal and marginal position corresponding to the heart (Fig. 1). After filtration and amplification, the signal was transmitted to a computer (see Santini et al., in press) in two alternative ways: (1) by a digital oscilloscope (Virtual Bench software), which allowed instantaneous and graphic visualization of heartbeats; or (2) by a Quick Basic program, which captured voltages every 0.1 s, throughout set periods of time, allowing long-term storage of data. We used the first method prior to each recording session to check the correct position of the sensor on the shell and the second for recording data during the experiments.

For each interval of continuous recording, a sequential file was automatically stored and exported for processing. Since we monitored the heartbeat of mussels up to the point of death, we expected to record arrhythmic or temporarily arrested heart rates. We therefore processed data by statistical determination of rhythmicity of the

heart rate. Autocorrelation analysis for time series data (Diggle 1990) was applied to each period of continuous recording to test whether heart rate was significantly periodical ($P < 0.001$). The periodicity, if any, was estimated by the particular time lag corresponding to the highest value of the autocorrelation coefficient. This procedure, therefore, allowed us to detect those recordings, near to the death point of mussels, for which heartbeat was either arrhythmic or absent. If periodicity was detected, time lag values of heartbeat frequency were converted to beats/min.

Methodological details for recording the radular activity of dogwhelks attacking mussels are reported in Parpagnoli & Chelazzi (1995) and Rovero et al. (1999). Piezoelectric transducers were fixed to the same valve bearing the heart rate sensor, close to the dorsal hinge (Fig. 1). The other valve was glued to a PVC base, facilitating video monitoring of behavioural patterns. This isolation and unnatural position of the prey does not alter the attack methods used by dogwhelks (see below) or the responsiveness of the mussels (Rovero et al. 1999). Data were stored on a computer and exported after each attack for processing. We obtained profiles of drilling activity, throughout the shell penetration process, quantified as the number of radular rasps per h, each rasp corresponding to a series of strokes of the radular teeth on the substratum.

Heart rate and drilling recordings were coupled with video monitoring of the animals by a camera placed beside the aquarium, connected to a time-lapse video recorder that stored one frame every 3 s.

Procedures and Data Analysis

Experiment 1: effect of predation threat on heart rate

To monitor cardiac rates during the normal feeding activity, we placed eight mussels equipped with heart rate sensors into the aquarium and sequentially recorded heartbeats for 1 h, divided into 60 s of continuous recording every 5 min. Mussels were first allowed to acclimatize to experimental conditions for about 30 min, until they assumed the gaping posture (i.e. open valves) indicating active ventilation (Bayne 1971) and heart function (Coleman 1974). After these trials, 50 dogwhelks were placed in the same aquarium inside a compartment of 10×10 cm made of plastic mesh and left for 12 h together with the mussels. We then monitored the mussels following the same procedure as described above. The experiment lasted 2 days.

We computed mean heart rate of each mussel for both treatments; values were analysed together with those for the same mussels under the first 3 h of predatory attack (see experiment 2). Means were compared by repeated measures ANOVA (Sokal & Rohlf 1995).

Experiment 2: heart rate under predatory attack

A mussel bearing both sensors was placed together with a dogwhelk into a compartment of 10×10 cm within the aquarium. We monitored each predatory sequence from the beginning of inspection to when the dogwhelk

abandoned the consumed mussel (the attack lasted about 90 h for bored hole attacks and 45 h for gape insertion attacks, see below for a description of attack methods). The predator-prey pair was then replaced with another. These experiments were run over a period of 10 weeks.

Data from video, drilling activity and heart rate monitoring were analysed and integrated as follows. From video monitoring, we classified attacks by method, according to whether the dogwhelk penetrated the mussel by drilling a hole through the shell (bored hole method) or by inserting its proboscis between the gaping valves (gape insertion method; see Rovero et al. 1999; Fig. 1). The behaviour of mussels was analysed for gaping rate (i.e. number of gapes per h, each gape being a closure of the valves followed by immediate reopening). Since for high gaping rates the exact values were not discernible, gaping rate was expressed on an ordinal scale: 0: non-gaping; 1: 1–9; 2: 10–19; 3: 20–49; 4: 50–99; 5: >100 gapes/h. When no, or little, gaping activity occurred, we noted whether the valves were kept open or closed.

Prey handling by dogwhelks consists sequentially of inspection, drilling and ingestion phases (Hughes & Dunkin 1984). Monitoring drilling activity allowed us to distinguish these phases (Rovero et al. 1999) and so to categorize the heart rate of mussels for each phase separately. The drilling phase was defined as the period characterized by radular activity and subsequent heart rate values were considered to represent the ingestion phase.

Heartbeats of mussels were recorded for 30 s every 15 min throughout the predatory attack and mean hourly values of heart rate were computed. We considered those periods for which no significant rhythmicity was detected by autocorrelation analysis to represent temporary cardiac pausing (heart rate=0) and we scored them separately as % pausing/h. The death of mussels was judged by the continuous recording of cardiac pausing. Individual profiles combining heart rate and % pausing were plotted together with behavioural profiles showing gaping rate and mutual position of valves (open or closed).

Profiles of heart rate were also pooled for all attacks, grouped by attack method. We used regression analysis to visualize trends and compared slopes by repeated measures ANOVA. To analyse differences between attack methods, we normalized variations in the length of attacks by dividing each attack into four equal temporal segments, for which we computed mean values of cardiac and behavioural parameters (heart rate, % pausing/h, index of gaping rate and fraction of hours with closed valves). Differences of these parameters between temporal segments and between attack methods were tested by repeated measures ANOVA, using the Huynh-Feldt epsilon to reduce degrees of freedom to accommodate for nonsphericity (Norušis/SPSS Inc. 1993).

Experiment 3: temporal dynamics

The design of experiments 1 and 2 was constrained by the available recording apparatus. Thus, heart rate could be continuously recorded for only one mussel at a time and even instantaneous readings, switching between subjects, could be taken for no more than eight mussels within an acceptable time period (5 min). Therefore,

pretreatment and treatment phases had to be run sequentially, as in experiment 1. We designed experiment 3 to test the independence of changes in heart rate and residence time of mussels in aquaria, by using a pretreatment period equalling the total duration of both experiments 1 and 2. The experiment also examined in greater detail the temporal dynamics of heart rate resulting from temporary exposure to dogwhelk effluent.

We placed eight mussels in an aquarium as in experiment 1, but in the presence of an empty cage. We monitored the heart rate of each mussel over 3 days, making instantaneous readings twice per day. We then placed 50 dogwhelks in the cage and made instantaneous recordings of heart rate once per h for the first 12 h, and once again at 24 h. We then removed the dogwhelks from the cage and changed the water. To maximize removal of effluent, untreated water was allowed to circulate in the aquarium for about 10 min. Heart rate was then monitored twice per day for 2 more days, as in the pretreatment phase. The experiment lasted 1 week.

A profile of mean heart rate per day was plotted for the pretreatment, treatment and post-treatment phases, together with a more detailed profile of heart rate during exposure to effluent. Values were compared by repeated measures ANOVA and by Wilcoxon's signed-ranks test (Sokal & Rohlf 1995).

RESULTS

The mean heart rate \pm SE was 23.10 ± 0.61 beats/min during normal feeding activity and 29.82 ± 0.91 beats/min in similar conditions but in the presence of effluent from dogwhelks (Fig. 2a). This variation was not followed by any variation in the gaping rate, since mussels kept the valves open almost constantly in both treatments. When in contact with the predator, the heart rate of the same mussels increased further, to 36.84 ± 1.59 beats/min, concomitant with an increase in the gaping rate (see below). Differences in heart rate between the three treatments were significant (repeated measures ANOVA: $F_{2,14}=65.90$, $P<0.001$). Figure 3 shows examples of heartbeat profiles, for one mussel under different treatments. The increase in heart rate from treatment 1 to treatment 3 was accompanied by an increase in the voltage amplitude of the signal.

In experiment 3, the heart rate of mussels did not vary significantly during the pretreatment phase (Fig. 2b; repeated measures ANOVA for 1–3 days: $F_{2,14}=2.38$, NS). After 1 h of exposure to dogwhelk effluent, the heart rate increased significantly (Fig. 2c; Wilcoxon test between the last h of pretreatment and the first h of treatment: $Z = -2.37$, $N_1=N_2=8$, $P<0.02$), showing a peak after 2 h and then stabilizing at a mean \pm SE of 31.15 ± 0.09 beats/min, without any significant variation until at least 24 h from first exposure to effluent (repeated measures ANOVA for 3–24 h: $F_{8,56}=1.62$, NS). As noted in experiment 1, mussels did not show any behavioural difference between pretreatment and treatment phases. When the effluent was removed, the heart rate decreased slowly, returning after 24 h to values not significantly different from those during pretreatment (Fig. 2b;

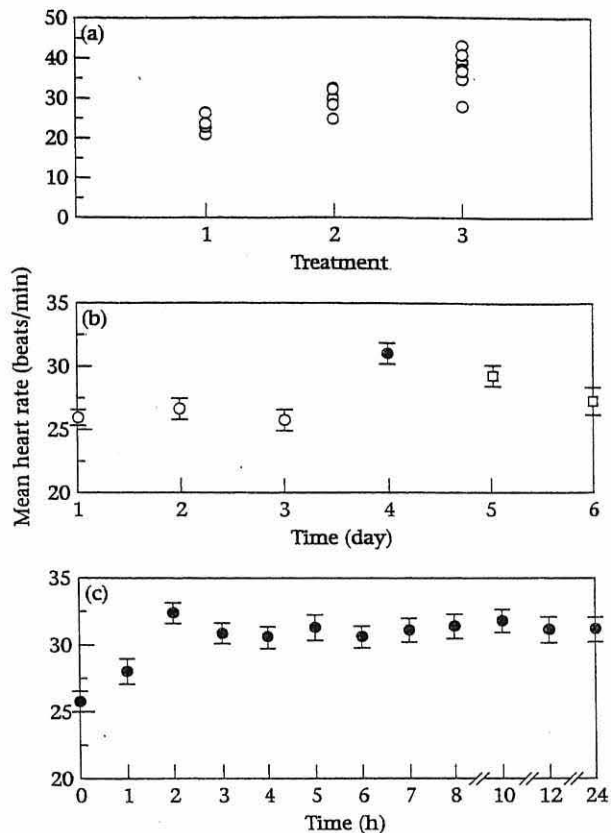


Figure 2. (a) Mean heart rate (beats/min) of eight mussels consecutively monitored under three treatments: (1) during 1 h of normal feeding activity; (2) during similar conditions but in the presence of effluent from dogwhelks; and (3) during the first 3 h under predation attack by dogwhelks. (b) Mean \pm SE heart rate pooled for eight mussels monitored for 3 days during normal feeding activity (○: pretreatment phase), during 1 day in the presence of dogwhelk effluent (●: treatment phase), and during 2 days after removal of dogwhelk effluent (□: post-treatment phase). (c) Mean \pm SE heart rate of the same mussels as in (b) in relation to time since exposure to dogwhelk effluent.

repeated measures ANOVA for 1–3 and 6 days: $F_{3,21}=2.85$, NS).

We monitored responses of mussels to nine gape insertion attacks and seven bored hole attacks by different dogwhelks. Further replication was prevented by the long duration of staging and observing each attack (in the order of 4 days per attack). Figure 4 shows profiles of cardiac and behavioural parameters for each method for two individuals. For mussels attacked by the bored hole method, the mean heart rate tended to increase throughout the attack and then showed a marked increase after the beginning of ingestion (Fig. 4a). The heart rate was significantly arrhythmic during most of the attack, only becoming arrhythmic or ceasing during the final 10 h. Gaping rate was high at the beginning of drilling (20–50 gapes/h), then stabilized at values of 10–20 gapes/h, and suddenly dropped to zero about 10 h after the beginning of ingestion. This last period was always characterized by closure of the valves.

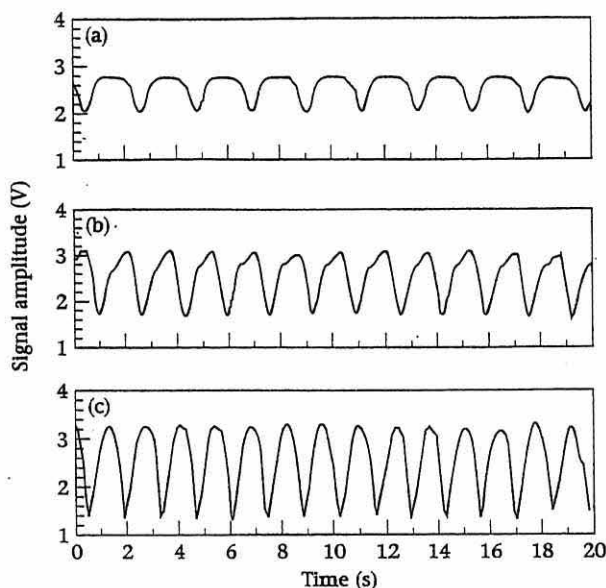


Figure 3. Heartbeat profiles, plotted for a period of 20 s, for a mussel monitored under the same three treatments as described in Fig. 2. (a) Treatment 1; (b) treatment 2; (c) treatment 3.

When dogwhelks used the gape insertion method of attack, the heart rate of mussels showed a more variable pattern between individuals, and in a few cases decreased throughout the attack (Fig. 4b). Temporary cardiac arrest (pausing) generally appeared relatively earlier than in bored hole attacks. Radular activity did not occur during most attacks by gape insertion and so handling phases could not be distinguished. Gaping rate was high during the first 5 h (50–100 gapes/h) and gaping ceased relatively early, concomitant with passively open valves and retraction of the mantle rim.

Hourly values of heart rate were pooled over a range of 22–50 h after the beginning of attack, for gape insertion and bored hole attacks, respectively (Fig. 5), as the mussels were generally killed within those periods. Regression analysis confirmed that under the gape insertion method the heart rate decreased slightly during the predation sequence (least squares linear regression: mean heart rate = $33.50 - 0.12$ h, $F_{1,20} = 10.18$, $R^2 = 0.33$, $P < 0.005$), whereas under the bored hole method the heart rate increased (mean heart rate = $30.79 + 0.07$ h, $F_{1,48} = 15.15$, $R^2 = 0.23$, $P < 0.0005$), the rates of change being significantly different between attack methods (repeated measures ANOVA: attack method \times time interaction: $F_{1,49} = 1.65$, $P < 0.05$).

The heart rate normalized along the temporal scale did not vary significantly either between temporal segments (Fig. 6a; repeated measures ANOVA: $F_{2,4,33.2} = 0.33$, NS) or between attack methods ($F_{1,14} = 0.01$, NS). In contrast, cardiac pausing increased over time, with higher values occurring during the last quarter, before mussels were killed (Fig. 6b; $F_{2,2,30.6} = 14.5$, $P < 0.001$). Cardiac pausing in mussels attacked by the gape insertion method was more frequent than in those attacked by the bored hole

method ($F_{1,14} = 9.36$, $P < 0.01$). Gaping rate decreased during the attack (Fig. 6c; $F_{3,42} = 49.14$, $P < 0.001$), differences between attack method being nonsignificant ($F_{1,14} = 0.12$, NS). The proportion of time when mussels had closed valves varied significantly between temporal segments during the attack (Fig. 6d; $F_{1,6,21.8} = 11.44$, $P < 0.01$) and was significantly higher with the bored hole method during the final segment ($F_{1,14} = 29.58$, $P < 0.001$).

DISCUSSION

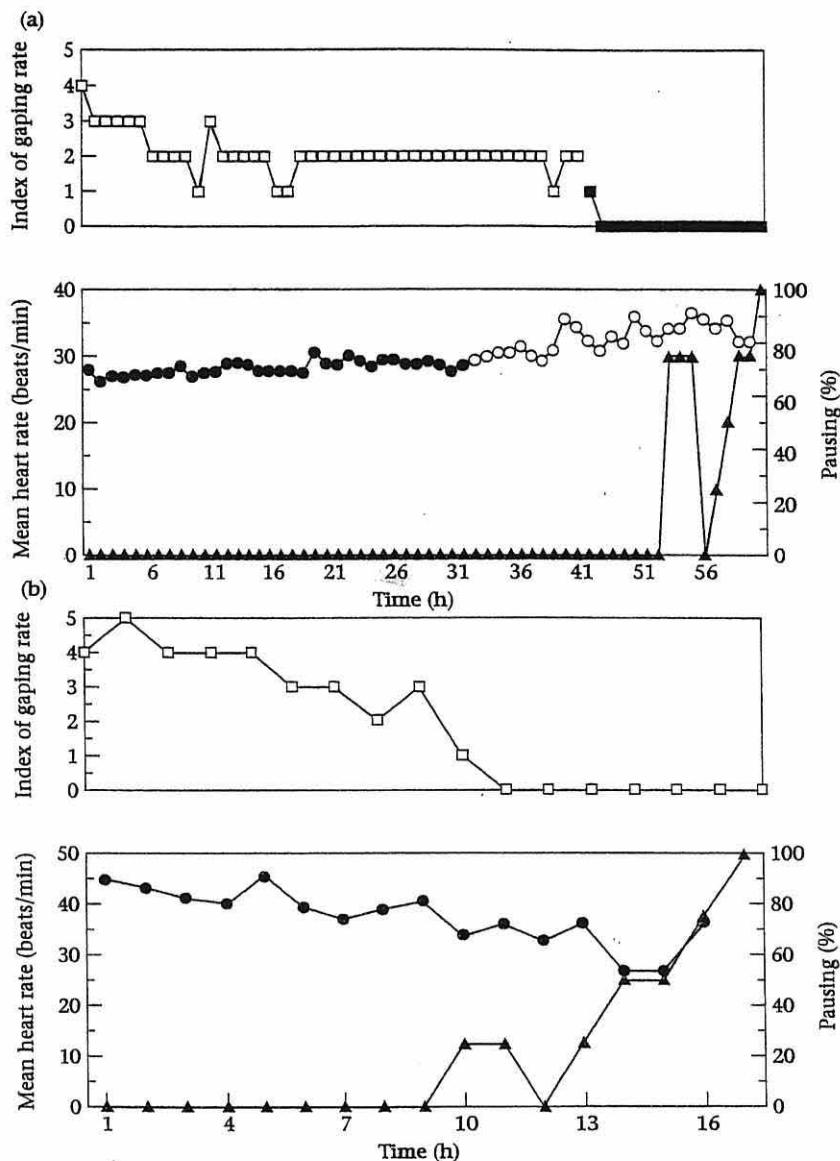
The noninvasive technique for monitoring heart rate has enabled, for the first time, an investigation of how risk of predation by dogwhelks is perceived by mussels and of how they respond when under attack.

We believe that the increase in heart rate of mussels after dogwhelks were put into the aquarium, in experiments 1 and 3, was due to an olfactory-mediated perception of predation threat (reviewed in Kats & Dill 1998). Residence time in the aquarium did not influence the heart rate of mussels, at least not over a period equivalent to the experimental one. We therefore discount time elapsing from pretreatment to treatment as a potentially confounding factor in our interpretation of the results. This conclusion is also clearly supported by the relatively sudden increase in heart rate after mussels were exposed to effluent from dogwhelks in experiment 3.

Mussels did not show signs of habituation while dogwhelks were present in the aquarium. Moreover, the heart rate remained elevated for a protracted period even after the olfactory cue had been removed. Prolonged refuging or vigilance in response to olfactory signals of predation risk has been noted in several taxa (Kats & Dill 1998). A continued response may be adaptive if the predator is likely to return soon after the olfactory cue attenuates.

Chemical cues from predators are known to induce behavioural responses of prey, such as reduced foraging activity in muricid gastropods (Palmer 1990; Vadas et al. 1994; Serra et al. 1997). In mussels, cardiac response to the perceived threat of predation did not appear to be associated with overt behavioural variation, particularly of gaping rates, but represented an alerted physiological state characterized by enhanced heart rate. Without appropriate data it remains unknown whether filtration and respiratory rates varied concomitantly. However, Bayne (1971) suggested that the low oxygen-carrying capacity of the blood of *M. edulis* is compensated by a large blood volume. This would provide an oxygen store that is large in proportion to the mass of respiring tissue. If so, enhancing circulation by increasing heart rate could reflect increased metabolic rate, which might allow the mussels to respond more effectively to a potential attack.

There is evidence that mussels can temporarily immobilize dogwhelks by using byssal threads (Petraitis 1987; Day et al. 1991), although dogwhelks can break free from byssal entrapment (Davenport et al. 1996). We did not observe this potential response of mussels to dogwhelk attack, probably because of the fixed position of our mussels. Nevertheless, the increased gaping rate shown by mussels under attack is likely to have a defensive



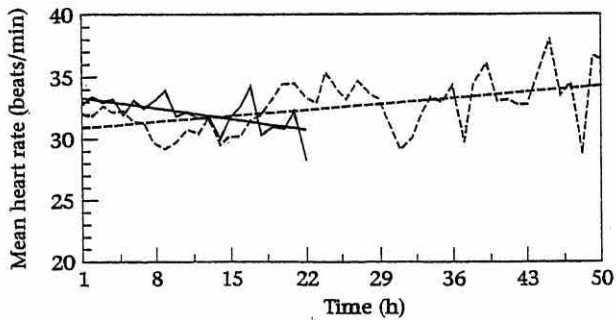


Figure 5. Mean hourly values and regression line of heart rate of mussels experiencing different attack methods by dogwhelks: penetration through the gaping valves (—) and penetration through a drilled hole (---). Values are plotted from the beginning of attack for a period of 22–50 h, for gape insertion and bored hole methods, respectively, since within that period the mussels were generally killed by dogwhelks.

At the beginning of the attack, cardiac responses of mussels were very similar irrespective of the penetration method used by dogwhelks. Nevertheless, when the entire predation sequence was examined, the responsiveness of mussels was clearly affected by attack method. Behavioural observations suggested that muscular paralysis occurred when mussels were attacked by dogwhelks using the gape insertion method. This result supports the hypothesis that *N. lapillus* is able to paralyse the prey by injecting toxins secreted by the accessory salivary glands (West et al. 1996). Paralysis also involved the heart

muscle, both lowering heart rate and causing cardiac pausing, this again matching pharmacological findings (Andrews et al. 1991; West et al. 1996).

Once the attack was initiated, mussels were always eventually killed; therefore it is difficult to assess whether the observed reactions could have a defensive function. The trend of increasing heart rate shown by mussels under drilling attack was concomitant with closure of the valves once ingestion by dogwhelks had begun. This response limits gaseous exchange, limiting oxygen supply to tissues (Marshall & McQuaid 1993) and so perhaps accounting for the progressively increasing cardiac frequency. Studies aimed at directly correlating cardiac responses with respiratory variables would be required to verify the consistency of these conclusions.

This study shows that noninvasive monitoring of heart rate, while being a reliable technique for physiological investigations (Depledge & Andersen 1990), can also enhance behavioural analysis, particularly when physiological states of animals need to be considered to assess effects of constraining factors.

Acknowledgments

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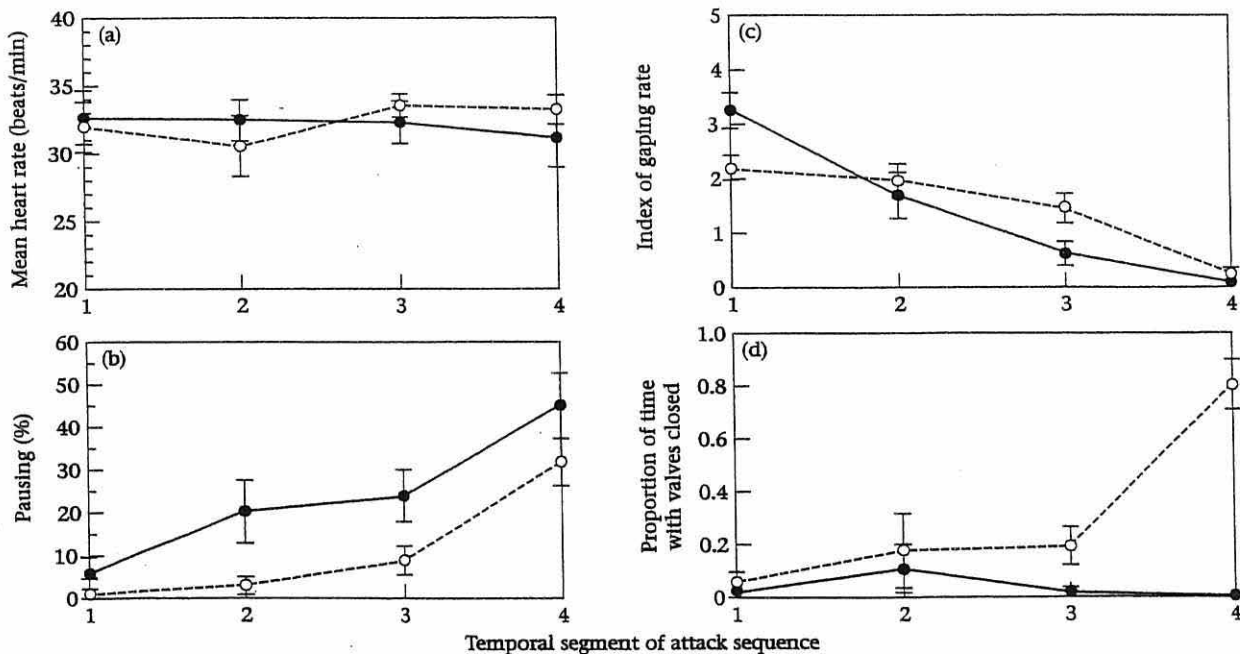


Figure 6. Mean \pm SE values of cardiac (a, b) and behavioural (c, d) responses of mussels to predation by dogwhelks. Variations in the length of attacks were normalized by dividing each attack into four equal temporal segments. Values are categorized for attack method by dogwhelks, i.e. penetration through a bored hole (○) or penetration between the gaping valves (●). See legend of Fig. 4 for a description of parameters.

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Estimating the energetic cost of fighting in shore crabs by noninvasive monitoring of heartbeat rate

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After establishing shore crabs, *Carcinus maenas*, individually in separate aquaria, we used a noninvasive infrared phototransducer to monitor their heartbeat rate continuously before, during and after fights with intruder crabs. We confirmed that heartbeat rate is a reliable indicator of oxygen consumption and then used it to estimate indirectly the energetic cost of fights differing in duration and intensity, and its dependence on prior residence and relative size of opponent. Prior residence in aquaria significantly increased the probability that crabs would initiate fights against intruders. The majority of fights were resolved by aggressive contacts, display being used extensively only against smaller intruders. Fights between evenly sized opponents and between residents and larger intruders involved almost continuous aggression, whereas fights with smaller intruders involved several shorter bouts of aggression. Fight duration was weakly correlated with the relative size of opponents. Heartbeat rate, measured only in residents, was elevated above resting levels throughout fights, hence energy expenditure during fighting increased linearly with fight duration. Contrary to expectation, heartbeat rate was not significantly influenced by relative size of the opponent or by the intensity of aggression. After fighting, heartbeat rate usually returned to resting levels within 30–60 min, recovery taking longer in fights against larger intruders, when the fight was always lost. We propose that prolonged elevation of heartbeat rate in residents that had lost to larger intruders represented a state of alertness, adaptive against impending risks of resource loss or injury.

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Agonistic behaviour is critically related to fitness because for most animals, while potentially endangering survival, it ultimately determines access to limited resources, particularly food and mates (Parker 1974; Archer 1988). It is generally assumed that natural selection optimizes the balance between potential benefits and costs experienced by contenders, so shaping the evolution of fighting strategies (Maynard Smith & Price 1973; Maynard Smith 1982). To develop this cost-benefit approach, it is essential to measure the fitness consequences of alternative behavioural acts (Riechert 1988). One of the most immediate consequences is metabolic cost and attempts to measure this in terms of aerobic and anaerobic metabolism during and after fights have been fruitful. The methods used, however, have either been

destructive (Thorpe et al. 1995), invasive and therefore potentially stressful (Smith & Taylor 1993), or unable to record opponents separately, whose metabolic rate therefore had to be assumed equal (Hack 1997). A noninvasive technique that allows the metabolic state of individuals to be monitored continuously should avoid most of the above problems, so widening scope and enhancing resolution for estimating costs of fighting.

Infrared monitoring of cardiac activity (Depledge & Andersen 1990) is a noninvasive technique widely applied to physiological problems (e.g. Aagaard et al. 1995; Aagaard 1996) and recently extended to behavioural experiments (Rovero et al. 1999). In the present study, we used this technique to monitor the heartbeat rate of shore crabs, *Carcinus maenas* L., continuously during fights and subsequent recovery. Having established a correlation between oxygen consumption and heartbeat rate, we used the resultant data to estimate the metabolic costs of alternative fighting strategies. To induce different fighting strategies we exploited the

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behavioural influences of prior residence (Enquist & Leimar 1987), which has not previously been studied in shore crabs but is well known in a wide variety of territorial animals (e.g. Figler & Einhorn 1983; Englund & Otto 1991), and relative body size (e.g. Maynard Smith & Parker 1976). Crabs are excellent model animals for this purpose, as they fight readily in the laboratory, show various intensities of aggression (Huntingford et al. 1995) and are sufficiently large and suitably shaped for successfully mounting external transducers (Depledge & Andersen 1990).

METHODS

Collection and Maintenance of Animals

We collected male shore crabs from the Menai Strait, North Wales, U.K., by using baited traps. Those with a hard exoskeleton and no missing or regenerating limbs were kept individually in holding tanks of 25 × 40 cm and 30 cm high, containing aerated sea water held at 14 ± 1°C under continuous illumination to reduce any variation in heartbeat rate caused by circadian rhythms (Naylor 1996).

Subjects were fed ad libitum with mussels, *Mytilus edulis*, for at least 1 week prior to experimentation. Preliminary observations revealed that after this period, 'residents' were strongly motivated to fight against 'intruders' transferred from other tanks. We chose male crabs of 5–6 cm carapace width to be residents and fitted them with sensors for measuring heartbeat rate (see below). Intruders were male crabs of various sizes, not fitted with sensors. We kept residents and intruders deprived of food for 24 h before each experimental session in order to standardize hunger level and associated aggressive motivation. We used crabs within 2 weeks of collection and kept them for 2 more weeks after experimentation to ensure that they were not in proecdysis (none was). Experiments were run from January to April 1999.

Monitoring Heartbeat Rate

Using cyanoacrylate glue, we fixed an infrared phototransducer (Depledge & Andersen 1990) to the carapace above the pericardial sinus. The signal from the transducer was transmitted to a computer. Heartbeat rate could either be displayed on a digital oscilloscope (Virtual Bench software) or continuously recorded by a Quick Basic program, capturing values at 0.1-s intervals and producing sequential files of voltages (see Rovero et al. 1999 for details). We used the oscilloscope to measure the heartbeat rate of residents prior to staging fights and the computer system to record data during behavioural trials. We compared the continuous heartbeat profiles with corresponding behavioural patterns. Lack of duplicate signal-processing equipment confined recording to one individual at a time. Experiments were therefore protracted and this prevented us from monitoring heartbeat rates of intruders.

Experimental Procedures and Data Analysis

Agonistic behaviour

We staged fights between paired opponents comprised of a resident crab (carapace width: $\bar{X} \pm \text{SE} = 54.57 \pm 0.46$ cm, $N=36$) and an intruder of similar (55.54 ± 0.99 cm, $N=18$), smaller (41.97 ± 1.00 cm, $N=18$) or larger size (70.70 ± 1.15 cm, $N=17$). In the first type of pairing, the size ratio of the smaller crab to the larger was 0.85–1.00. Size ratios within this range do not allow accurate prediction of fight outcome either in *C. maenas* (Sneddon et al. 1997a) or in other species (Huntingford et al. 1995). We used different batches of residents for evenly size-matched and unevenly matched pairings. Residents in evenly size-matched pairings were each subjected to one fight. Residents in unevenly matched pairings were each subjected to two fights, one with a larger and one with a smaller intruder, staged in random order with an interval of 2–3 days between fights. By adopting this mixed design, we compromised between the advantage of repeated measures (control of individual variation) and that of statistical independence.

We used a video camera above the aquarium to film behavioural interactions. We also observed behaviour directly through openings 10 cm in diameter in black polythene sheets hung around the tank to screen subjects from disturbance. We recorded the heartbeat rate of each resident continuously for 10 min before the intruder was introduced. We then slowly introduced the intruder through an opening in the polythene sheet and released it 20–30 cm from the resident. Handling of the intruders, although careful, may have reduced readiness to fight. Any such effect, however, would have reinforced the intended rank order of aggressiveness between residents and intruders. Moreover, it should have had no differential effect regarding size of the intruder. We monitored behavioural interactions for 18 min, within which time fights were always resolved. We then removed the intruder, using the reverse procedure, and recorded the heartbeat rate of the resident for a further 18 min. Preliminary trials had shown that heartbeat rate decreased to resting levels within this time period. The single-channel input of the recording apparatus forced us to run trials sequentially.

Control

To test whether heartbeat rate of the resident could have been altered simply by the process of introducing or removing the intruder, we followed the same procedures as for the experimental treatments except that an empty hand was introduced and withdrawn from the aquarium. We randomized the order of experimental and control treatments for each crab. Residents used for size-matched pairings therefore each experienced one experimental and one control treatment, in random order. Those used for unevenly sized pairings experienced two experimental plus a control treatment, again in random order.

Behavioural patterns and heartbeat rate

Following previous studies (e.g. Smith et al. 1994; Sneddon et al. 1997b) we timed behavioural interactions

from the moment the initiator adopted an aggressive display or from the moment its approach was followed by retreat of the opponent. The fight lasted until one crab, the winner, elicited the last in a series of consecutive retreats from its opponent. Usually, no further aggressive interactions occurred once the loser had made two or three retreats within about 30 s of each other. Any such re-engagements, however, were included in the measurement of fight duration.

We classified aggressive behavioural patterns as display (extended chelipeds) or contact (pushing, grasping or striking with chelipeds). Interactions could involve unilateral or bilateral acts: in the former, only one crab performed aggressive acts and the other retreated submissively, whereas in the latter both crabs engaged reciprocally in aggression. We therefore categorized interactions as follows: unilateral display (by resident or intruder), bilateral display, unilateral contact (by resident or intruder), bilateral contact.

We used chi-square tests to compare the distributions of fight initiation and fight outcome between residents and intruders and to compare the frequencies of behavioural patterns across treatments with similarly sized, smaller and larger intruders.

We estimated 'resting' heartbeat rate for each resident as the mean recorded heartbeat rate during periods of quiescence prior to the introduction of the intruder. Similarly, 'fighting' heartbeat rate was taken as the mean heartbeat rate during the fight. We calculated 'excess' heartbeat rate during fighting (Smith & Taylor 1993) as $((\text{fighting heartbeat rate} - \text{resting heartbeat rate}) \times \text{fight duration}) / (\text{resting heartbeat rate})$. This corresponds to the time (min) that would be required for the number of beats recorded during the period of excess to have been executed at resting level. Since resting heartbeat rate did not differ significantly when crabs were paired against smaller or larger intruders (see Table 3 in the Results; paired t test: $t_{15} = -0.19$, NS), we used the mean resting heartbeat rate of each crab to compute excess heartbeat rate. Analogous methods were used to calculate excess heartbeat rate during recovery (see Results for further explanation).

We used paired t tests or Wilcoxon signed-ranks tests in the case of non-normality to compare behavioural and cardiac parameters of residents when fighting smaller and larger intruders. We used t tests for independent samples or Mann-Whitney tests to compare parameters between evenly size-matched and unevenly matched fights. To compare mean heartbeat rate between experimental and control treatments, we used paired t tests for evenly size-matched pairings and repeated measures ANOVA for unevenly matched pairings. The same procedures were used to compare mean heartbeat rate after removal of the intruder with that during the control treatment.

Respirometry

We measured oxygen consumption rate with closed respirometers ca. 300 ml in volume. We removed small samples of water from the respirometers to measure oxygen partial pressure (see below). To determine the relationship between heartbeat rate and respiration rate,

we measured both variables in crabs initially disturbed by handling and again during recovery. We took crabs fitted with phototransducers and held them below the water surface for 2 min, allowing the legs to move freely. This procedure disturbed the crabs sufficiently to elevate heartbeat rate. We immediately placed each crab in a respirometer and recorded oxygen uptake and heartbeat rate for 10 min. We then opened the respirometer to the surrounding sea water to regain ambient oxygen tension and allowed the crabs to settle for 2 h, during which time heartbeat rate returned to resting levels. We resealed the respirometers and measured oxygen uptake over a further 10 min, while the crabs were still in an undisturbed state.

Oxygen partial pressure was measured with an oxygen electrode (Radiometer E5047) held in a cuvette maintained at 14°C and connected to an oxygen meter (Strathkelvin Instruments, Glasgow, U.K.). We calculated oxygen uptake rates according to Dejours (1981), using oxygen solubility coefficients corrected for temperature and salinity. The resultant data were plotted against corresponding changes in heartbeat rate. We used linear regression to analyse the relationship between heartbeat rate and oxygen consumption rate.

All statistical tests used two-tailed probability criteria at $\alpha=0.05$.

Ethical note

On local shores, *C. maenas* forage actively in daylight (Cunningham 1983) and continuous illumination should not have caused undue stress while crabs were kept in the laboratory. Aggression did not escalate during fights and opponents never inflicted injury upon one another. Afterwards, all crabs were returned to the collection site. We believe that, in comparison with other methods, the technique used in this study minimizes the potential stress to subjects. Fitting the phototransducer involved removing the crab from the tank, cleaning the carapace and waiting 1–2 min for the glue to dry. The behaviour of crabs did not appear to be altered by the presence of the sensors and wires, whose weight of 1.2 g represented about 3% of total body mass. The transducer was cubic, of width 0.5 cm, fitted to a wire of gauge 0.5 mm. The loosely suspended wire appeared not to impede movement of the crab. The transducer was removed before the crab was returned to the collection site.

RESULTS

Behavioural Strategies

Forty-eight fights were recorded. Resident crabs initiated fights significantly more frequently than intruders (Table 1; chi-square test: $\chi^2=14.08$, $P<0.01$). The proportion of initiations by residents decreased from 89% when fighting smaller intruders to 64% when fighting larger intruders. The chance of winning depended primarily on the size of the opponent: in unevenly matched fights, the larger crab always won, regardless of residence status. When evenly size-matched, residents won 69% of

Table 1. Number of interactions between resident crabs and intruders of smaller, similar and larger size, respectively

	Relative size of intruder		
	Smaller	Similar	Larger
Resident initiated	16	12	9
Intruder initiated	2	4	5
Resident won	18	11	0
Intruder won	0	5	14

The initiator is the crab that first performed aggressive behaviour or whose approach was followed by the retreat of the other crab. The winner is the crab that elicited a series of consecutive retreats from the other crab.

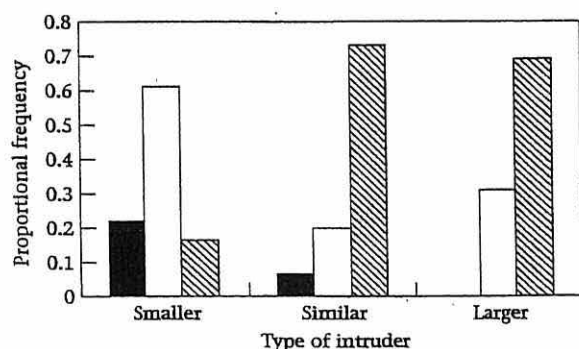


Figure 1. Proportional frequencies of different behavioural acts during fights between resident crabs and intruders that were smaller, similar in size or larger than the resident. ■: Unilateral display by the resident; □: unilateral contact by the resident; ▨: bilateral contact.

fights, but probably owing to the small sample size, this was not significantly different from random expectation (Table 1; $\chi^2_1=1.17$, $P=0.28$).

The majority of fights were resolved by unilateral or bilateral contact by the resident, with a minor proportion resolved by unilateral display by the resident, usually against smaller intruders (Fig. 1). Only one fight, between evenly size-matched crabs, was resolved by bilateral display and only one was resolved by unilateral contact, performed by the larger intruder. Although fights involving bilateral contact (grasps and strikes) were more likely to start by unilateral contact, in other cases intense bilateral contacts were performed without any previous displaying. There was, therefore, no apparent escalation in the intensity of aggression. The distribution of behavioural patterns was significantly different across the three types of pairings (chi-square test: $\chi^2_2=13.30$, $P<0.02$), bilateral contact being the dominant pattern both in size-matched pairings and in those with larger intruders. Consequently, different behavioural patterns were associated with different outcomes. When the resident won, unilateral contact occurred more frequently than bilateral contact, but the reverse was true when the resident lost ($\chi^2_2=9.94$, $P<0.01$). Fights involving bilateral

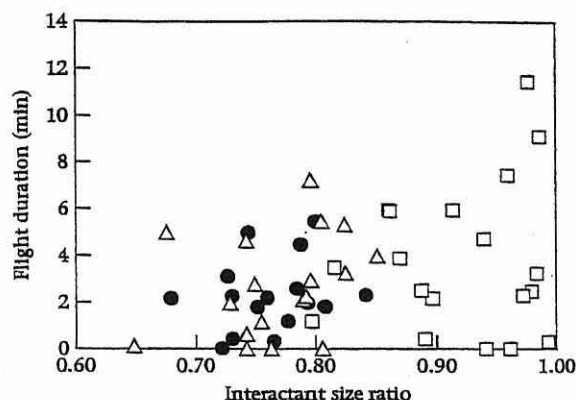


Figure 2. Duration of aggressive interactions between resident crabs and intruders, plotted according to the ratio between the carapace width of the smaller crab to that of the larger. ●: intruder smaller than resident; □: intruder similar in size to resident; △: intruder larger than resident.

contact were the most intense, but did not result in injury.

In most interactions between evenly size-matched crabs and between residents and larger intruders, fighting progressed as an almost continuous bout of aggression. Seven out of 18 fights against smaller intruders consisted of two to three bouts, in which the resident pursued the intruder around the aquarium. Fight duration was weakly correlated with the size ratio of opponents (Fig. 2; Pearson correlation: $r_{51}=0.32$, $P<0.02$). It was greater for fights between residents and smaller intruders than for fights between evenly size-matched opponents (Table 2; t test: $t_{28}=-2.47$, $P<0.03$, marginally nonsignificant after Bonferroni correction). Fight duration between residents and larger intruders was not significantly different from those of the other types of pairings. Moreover, durations of fights won and lost by residents were not significantly different either for unevenly size-matched pairings (above) or for evenly size-matched pairings (resident won: 4.89 ± 1.07 min; resident lost: 3.68 ± 0.70 min; t test: $t_{14}=0.73$, $P=0.48$).

Heartbeat Rate and Respiration Rate

Variation in heartbeat rate

Figure 3 shows heartbeat profiles for one of the fights. Resting heartbeats were arrhythmic, with frequent periods of pausing resulting in highly variable heartbeat rates ranging from 20 to 60 beats/min (Figs 3, 4). At the moment the intruder was introduced, heartbeat rate of the resident crab generally paused for about 10 s, before increasing rapidly to values between 80 and 120 beats/min. Subsequently, the highest heartbeat rates were recorded both during fighting and during the immediate postfighting stage, although the initial bout of aggression was often associated with an immediate decrease in heartbeat rate (Fig. 4).

Figure 5 shows the general trends, revealed by pooling data among residents. Heartbeat rate reached maximum

Table 2. Cardiac and respiratory parameters of resident crabs fighting against intruders of similar, smaller and larger size, respectively

	Smaller	Similar	Larger
Fight duration (min)	2.40±0.38 (14)	4.51±0.76 (16)	3.28±0.53 (13)
Excess HR during fighting (min)	4.4±1.1 (14)	6.6±1.6 (16)	5.3±1.2 (13)
Excess O ₂ demand during fighting (μmol O ₂)	2.38±0.52 (14)	3.63±0.80 (16)	3.35±0.66 (13)
Predicted recovery time (min)	29.36±5.17 (11)	59.9±20.2 (15)	62.0±12.2 (10)
Excess HR during recovery (min)	29.6±12.7 (11)	28.8±9.4 (15)	37.90±11.3 (10)
Excess O ₂ demand during recovery (μmol O ₂)	9.83±2.89 (11)	13.23±4.11 (15)	16.49±4.27 (10)
Total excess HR (min)	34.9±12.9 (11)	34.7±10.2 (15)	43.8±11.1 (10)
Total excess O ₂ demand (μmol O ₂)	12.47±3.05 (11)	16.72±4.51 (15)	19.75±4.11 (10)

HR: Heartbeat rate; see text for explanation of parameters. Data are means±SE; sample sizes are in parentheses.

values within 2–3 min of the appearance of the intruder, corresponding to the initiation of fighting. It remained elevated for various lengths of time, broadly reflecting differences in fight duration. This temporal variation was considered in the calculation of excess heartbeat rate (see below). Heartbeat rate decreased after the resolution of fights, slightly increasing again for 1–2 min after removal of the intruder (see below for analysis of the recovery phase). Mean heartbeat rate profiles did not vary significantly between crabs that fought against intruders of similar, smaller and larger sizes (Fig. 5, Table 3). Therefore, since fights against smaller and larger intruders had opposite outcomes, mean heartbeat rate did not differ between winners and losers. Mean heartbeat rate did not vary consistently between resident crabs fighting by bilateral contacts ($\bar{X} \pm SE = 87.82 \pm 3.11$, $N=21$), unilateral contacts (93.43 ± 3.84 , $N=15$), or unilateral displays (84.60 ± 9.07 , $N=5$).

Heartbeat rate of resident crabs during fighting was significantly higher than during control treatments (Table 3; evenly size-matched opponents, paired t test: $t_{15}=8.23$, $P<0.001$; unevenly matched opponents, repeated measures ANOVA: $F_{2,24}=18.52$, $P<0.001$). In contrast, mean heartbeat rate after removal of the intruder was not significantly different from control values (Table 3; evenly size-matched opponents: $t_{15}=1.95$, NS; unevenly matched opponents: $F_{2,30}=0.86$, NS).

Excess heartbeat rate

Excess heartbeat rate during fights was not significantly different between pairings with smaller and larger intruders (Table 2; Wilcoxon signed-ranks test: $Z=0.62$, $N=11$, NS; paired measurements were successfully obtained for only 11 residents). Although mean excess heartbeat rate was higher for crabs fighting against evenly size-matched opponents than against smaller or larger opponents (Table 2), the differences were not statistically significant (Mann-Whitney test: smaller intruders: $U=207.0$, $N_1=16$, $N_2=14$, NS; larger intruders: $U=174.0$, $N_1=16$, $N_2=13$, NS). Excess heartbeat rate during fighting was correlated significantly with fight duration both for winners and for losers, as expected since the former parameter is not independent of the latter (Pearson correlation: $r_{23}=0.81$, $P<0.001$ for winners, and $r_{15}=0.70$, $P<0.003$ for losers).

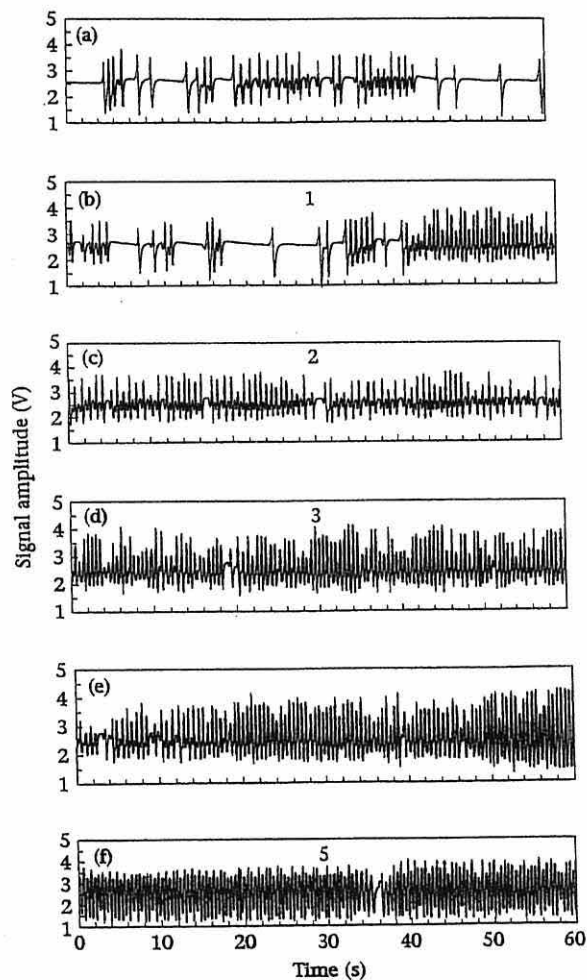


Figure 3. Continuous recording of mean heartbeat rate during successive phases of a fight between a resident and similarly sized intruder. (a) Undisturbed condition; (b) introduction of the intruder; (c)–(f) fighting bout. 1: Point of introduction of intruder; 2: initiation of fighting by the resident, followed by unilateral display and contact; 3: retreat of the intruder; 4: re-engagement by the resident and phase of bilateral contact; 5: retreat by the intruder. This fight was won by the resident.

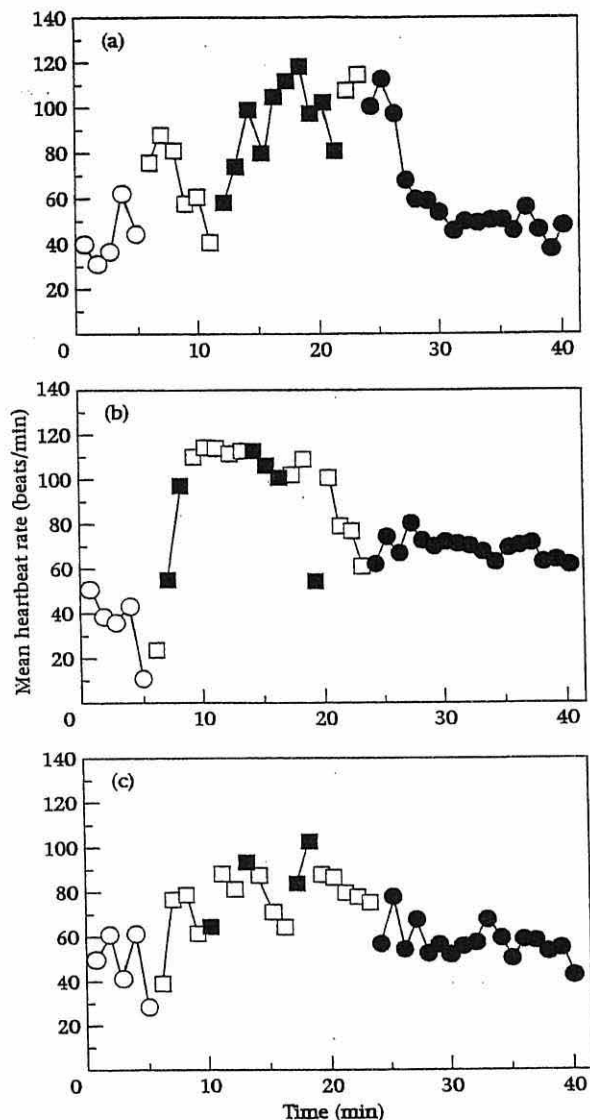


Figure 4. Profiles of mean heartbeat rate during successive phases of fights between resident crabs and intruders of different relative size: (a) smaller, (b) similar and (c) larger. \circ : Undisturbed conditions, before introduction of the intruder; \square , \blacksquare : presence of the intruder, divided into periods of fighting (\blacksquare) and nonfighting (\square); \bullet : period after removal of the intruder.

After fighting, heartbeat rate generally declined smoothly to resting levels (Fig. 5). Removal of the intruder, however, slightly raised the heartbeat rate for 3–4 min, similar to the effect of the control treatment. Consequently, recordings during the first 4 min after removal of the intruder were omitted from data analysis. Lines of best fit were obtained by linear regression after logarithmic transformation of time. By extrapolating from these regressions, we predicted the time that would be taken for heartbeat rate to return to resting levels, as

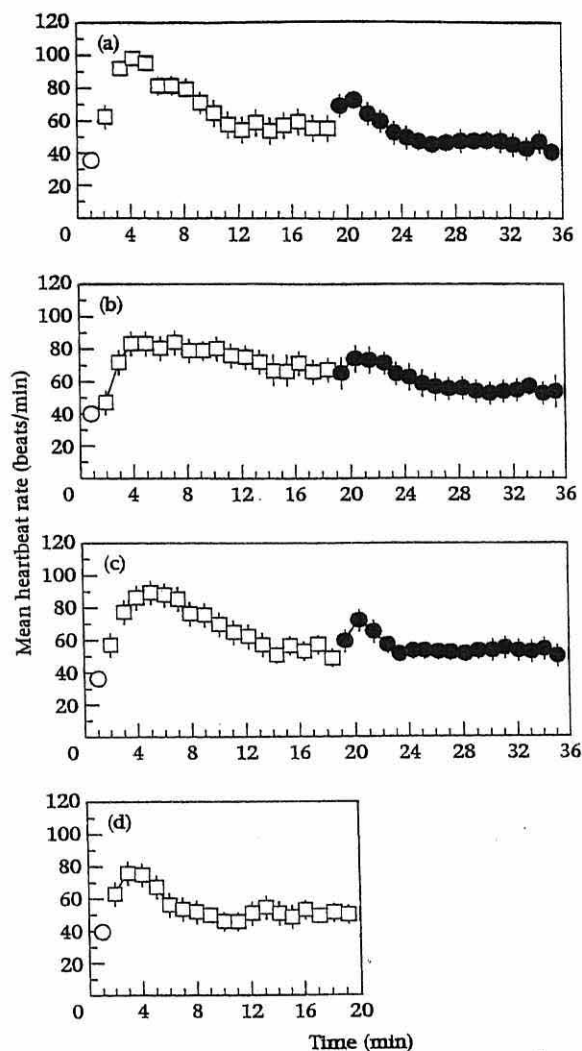


Figure 5. Temporal profiles of mean heartbeat rate \pm SE, each obtained by pooling data for different resident crabs, during successive phases of fighting with intruders of different relative size: (a) smaller, (b) similar and (c) larger size, respectively. The same resident crabs were used for fights (a) and (c). \circ : mean heartbeat rate during 10 min prior to the introduction of the intruder in the tank; \square : period during which the intruder was inside the tank; \bullet : period from removal of the intruder. Profile (d) records the mean heartbeat rate, pooled for all crabs, during undisturbed conditions (c) and control treatment (\square).

a measure of recovery time from fighting. Predicted recovery time was highly variable, although for 20 out of 36 crabs with significant regressions, recovery was completed within 30 min of fight resolution. Mean predicted recovery time was ranked in the order: pairings with smaller intruder < evenly size-matched pairings < pairings with larger intruder (Table 2), but pairwise comparisons were not statistically significant. Although overall difference in ranking was not statistically significant

Table 3. Heartbeat rate (beats/min) of resident crabs during different phases of the fight against intruders of similar, smaller and larger size, respectively

	Similar	Smaller	Larger
Resting	39.49±3.18 (16)	36.87±4.34 (16)	37.96±4.90 (16)
Intruder in tank	72.85±4.85 (16)	74.95±4.88 (16)	73.85±5.40 (16)
Fighting	86.43±4.45 (16)	91.22±4.00 (14)	91.45±5.28 (13)
Intruder removed	59.20±6.00 (16)	59.06±6.53 (16)	58.57±5.96 (16)
Control	50.50±3.57 (16)	51.96±6.01 (16)	

Resting: 10 min prior to the introduction of the intruder. Intruder in tank: the total period of 18 min during which the intruder was in the tank along with the resident. Fighting: the phase of aggressive interaction. Intruder removed: the period of 18 min after removal of the intruder. Control: the period of 18 min after disturbance of the resident by the introduction of the observer's hand. The same crabs were used for fights against smaller and larger intruders, whereas different crabs were used for fights against intruders of the same size. Data are means±SE; sample sizes are in parentheses.

(Kruskal-Wallis test: $\chi^2=4.35$, NS), mean recovery time was significantly different between pairings of residents with smaller and larger intruders (paired t test: $t_8=2.53$, $P<0.05$). From data pooled across pairing categories, predicted recovery time of residents was 41.4 ± 14.6 min ($\bar{X} \pm SE$) for winners and 64.9 ± 14.6 min for losers. Excess heartbeat rate during recovery, computed by integrating the recovery function over the predicted recovery time, did not vary significantly with size of the intruder (Table 2) and was not correlated significantly with fight duration, either for winners (Pearson correlation: $r_{18}=0.08$, NS) or for losers ($r_{10}=0.31$, NS).

Total excess heartbeat rate was estimated as the sum of excess heartbeat rate during fighting and recovery and therefore increased with the relative size of opponents (Table 2), although pairwise comparisons were not statistically significant (repeated measures ANOVA: $F_{1,8}=3.57$, $P=0.10$, observed power=0.38). Again, this parameter was not significantly correlated with fight duration, either for winners ($r_{18}=0.19$, NS) or for losers ($r_{10}=0.37$, NS).

Metabolic demand

Heartbeat rate was linearly related to oxygen consumption rate (Fig. 6). The regression, O_2 consumption ($\mu\text{mol } O_2/\text{min per g}$) = $0.00555 + 0.00035 \times \text{heartbeat rate}$, $F_{1,24}=21.66$, $R^2=0.47$, $P<0.001$, was used to predict oxygen demand from excess heartbeat rate during fighting and recovery (Table 2). The sum of these values gave the total excess oxygen demand resulting from fighting. Mean total excess oxygen demand was weakly associated

with the size of the opponent (Table 2), the difference between fights with smaller and larger contenders being marginally nonsignificant prior to Bonferroni correction (paired t test: $t_8=-2.18$, $P=0.06$; paired measurements were successfully obtained for only nine residents).

DISCUSSION

Behavioural Strategies

As intended, the two experimentally controlled asymmetries between opponents produced fights differing in duration and intensity. Residents were slightly more likely to initiate fights than intruders and the relative size of opponents clearly influenced fight duration and behavioural content. Fights tended to be longer and bilateral contacts more frequent when opponents were closely matched in size, as reported for other crabs (e.g. Glass & Huntingford 1988; Smith et al. 1994). Compared with fights against evenly sized opponents, duration and intensity were reduced more in fights against smaller than against larger intruders. This asymmetry reflected greater persistence of the resident, rather than aggressiveness of the larger intruder. Fighting was therefore intensified when the risk of losing ownership was increased by greater size of the opponent. Having obtained fights differing in quality, we could then examine any associated differences in metabolic cost.

Energetic Costs of Fighting

Heartbeat rate associated with aggressive behaviour was elevated throughout fights. Estimated energetic cost during fighting therefore increased with fight duration, as reported for other crabs (Smith & Taylor 1993) and for insects such as the house cricket, *Acheta domesticus* (Hack 1997). Contrary to expectation, however, the elevation in heartbeat rate did not vary according to the type of fighting behaviour adopted. Moreover, although the longer and more intense fights between evenly size-matched opponents were associated with higher excess heartbeat rate, this was compensated by a shorter recovery time compared with fights involving larger

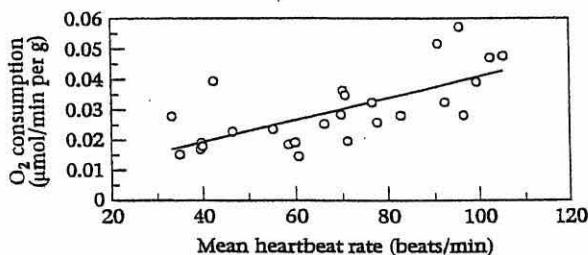


Figure 6. Relationship between oxygen consumption and heartbeat rate of crabs monitored before and after 2 h of exercise.

opponents. As a result, the total energetic cost was not significantly different in the two cases. Similarly, Smith & Taylor (1993) found that the energetic cost of fighting was not correlated with the ordinal intensity of fights, even though more intense interactions involved higher rates of oxygen consumption. They suggested that the relationship between fight characteristics and energetic cost is likely to be complicated by the permutations of different behavioural acts that may be involved. This is supported by our data, which represent a greater relative size range of opponents and associated variety of behavioural content and fight duration. Nevertheless, our data must be interpreted cautiously since the regression of oxygen consumption rate on heartbeat rate (Fig. 6) accounted for only 47% of the variance, perhaps weakening the power of our analysis to detect small differences in energetic cost associated with fighting intensity.

As the average elevation in heartbeat rate during the relatively short period monitored after fighting did not differ significantly from that prior to fighting, it is reasonable to assume that fighting is not an energetically expensive activity. This supports previous reports that fighting is much less expensive than enforced exercise (Sneddon et al. 1999) and has no adverse effect on subsequent activities (Thorpe et al. 1995). We found, however, that resident crabs that had fought against larger intruders took longer to recover than when they had fought against smaller intruders. This result cannot be adequately explained by the intensity and duration of fighting, which were highest for evenly size-matched contests. Instead, it is more likely that metabolic cost to the resident is ultimately determined by perceived risks of losing ownership or of injury, both of which are likely to increase with size of the opponent and may engender a prolonged state of alertness (see Austad 1983). Field observations corroborate this argument. Cunningham (1983) filmed shore crabs, unconstrained on the flooding tide, competing for access to a patch of mussels. Mean residence time was longer for larger crabs, who frequently used physical contact to fight intruders of similar size and elicited retreat from smaller crabs by using less intense aggressive activity without physical contact. Smaller crabs fed in an opportunistic manner, quickly visiting the patch and then immediately retreating. The prolonged alerted state of smaller losers, shown in the present study, might therefore be an adaptive response anticipating further engagement with superior opponents. An analogous, presumably adaptive state of alertness has been revealed by heartbeat recordings in mussels threatened by risk of predation (Rovero et al. 1999).

In conclusion, the noninvasive, externally mounted infrared phototransducer provided a satisfactory method of measuring the heartbeat rate of crabs, which itself was a good indicator of their aerobic metabolic rate. Metabolic cost was weakly determined by duration of the fight and appeared to be largely independent of its behavioural content or intensity (c.f. Hack 1997, but see also Smith & Taylor 1993). Moreover, greater size of the intruding opponent was accompanied by prolonged recovery and therefore greater total energy cost of fighting (Table 3), independently of fight duration or

intensity. Although tentative, owing to limited power of the statistical test, this result contradicts the game-theoretical prediction that costs should be higher when contests are more evenly matched (e.g. Maynard Smith & Parker 1976; Parker & Rubenstein 1981; Enquist & Leimar 1983). On the other hand, the prolonged elevation of metabolic rate may adaptively prepare the individual for further engagement if larger size of the intruder indicates risk of injury and/or loss of ownership. Another, complementary, interpretation is that residents fought harder against larger intruders because they perceived that greater effort was required to defend the resource successfully. Supporting evidence is confined to the weak correlation between fight duration and size of the intruder.

Crabs are proving to be excellent models for testing theories on fighting behaviour and this should be enhanced by measuring the energetic costs of alternative behaviours under a wider range of asymmetries and risks. For such purpose, noninvasive monitoring of heartbeat rate will be of great advantage.

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